The synthesis of some new heterocyclic analogues of the beta-lactam antibiotics

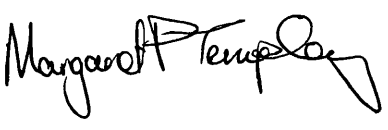
Templey, Margaret Patricia

Award date:
1988

Awarding institution:
University of Bath

THE SYNTHESIS OF SOME NEW HETEROCYCLIC ANALOGUES OF THE BETA-LACTAM ANTIBIOTICS.

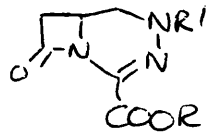
submitted by Margaret Patricia Templey
for the degree of PhD
of the University of Bath
1988



ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

CORRECTIONS

p7: structure labelled "2-aza-3-aza-1-dethiacephems" should be as shown below:



p17: footnote d) should read "$LiN(SiMe_3)_2$"

p18: line 32 should read "the 1-carbacephem analogue (60)"

p63: footnote h) should read "hν, $PhCMe_2O_2CNHNH_2$"

p89: line 9 should read "21% yield"

p116: line 10 should read "was not the starting material (230)."

p134: line 16 should read "Preparation of di-t-butyl fumarate (315)."

p135: line 1 should read "Preparation of di-t-butyl tartrate (316)."

p139: lines 7 & 8 should read "Preparation of t-butyl-(2-benzyl-6β-phenoxyacetamido-2-aza-1-thiapenicillanate) (329)"

p143: lines 4 & 5 should read "gave 8mg product (343) (3 %)"

CONTENTS

SUMMARY

Synthetic approaches towards novel analogues of the β-lactam antibiotics are discussed. The compounds studied are analogues of cephalosporins, penicillins and penems in which the carbon atom at position 2 of the bicyclic system is replaced by a nitrogen atom. These target compounds possess the structural elements required for antibacterial activity of β-lactams, and also possess unique reactivity and electronic factors. Hence these compounds are interesting as part of the search towards novel antibacterial agents.

The synthesis of the first example of a new class of cephalosporin analogues, potassium [2-phenyl-7β-phenylacetamido-2-aza-1-thiacephalosporanate] is described, and low-level antibiotic activity of this compound against Gram positive organisms has been demonstrated.
This compound was synthesised using similar methodology to that employed in a recent Hoechst synthesis of 7-unsubstituted 2-aza-1-thiacephems, and involved cyclisation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-phenylamidobut-2'-enoate)] azetidin-2-one. This intermediate was prepared in 6 steps from penicillin G.

Approaches towards the novel 2-aza-1-thiapenams were studied. Two complementary strategies were investigated: firstly involving cyclisation of (3-phenoxyacetamido-4-(2'-dithiobenzothiazolyl)azetidin-2-one)-yl-1-aminoacetates prepared from the corresponding known 1-hydroxyacetates. The second approach involved reaction of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)azetidin-2-one with imines derived from glyoxylic esters to produce intermediate 1-aminoacetates which were then cyclised as above. The products obtained in these studies exhibited a lack of stability, a property expected of this highly-strained, reactive bicyclic system.

Finally, approaches to the 2-aza-1-thiapenems were studied. The reaction of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)azetidin-2-one with cyanoformate esters were studied, but it was found that the azetidinone nitrogen was not sufficiently nucleophilic to attack at the cyano group of the cyanoformate. The said cyano group was activated by conversion to a thiooxamimidate, but nucleophilic attack still did not take place. However, a small amount of compound thought to be a 2-aza-1-thiapenem was isolated during the attempted preparation of 3-(phenoxyacetamido-4-(2'-dithiobenzothiazolyl)azetidin-2-one)-yl-1-azidoacetate. As expected, the isolated derivative was of limited stability.

1 INTRODUCTION.

The discovery of penicillin by Fleming in 1929[1] and subsequent development work by Howard Florey and Ernst Chain is well known to all with an interest in medical history.[2,3] In the 1940's various groups were engaged in the elucidation of the structure of the penicillin molecule, culminating in the X ray crystallographic analysis of penicillin G in 1945.

Also in 1945, Giuseppe Brotzu, a Sardinian Professor of Bacteriology, found that a strain of Cephalosporium acremonium produced antibiotic material. He had isolated this organism from the sea near a sewage outfall at Cagliari, believing that antibiosis might have a role in the "self purification" of sewage. Brotzu's discovery was a timely one, as penicillin-resistant staphylococci were becoming prevalent in hospitals, and it turned out that the cephalosporins,[4] with their broad spectrum of activity, were to provide the answer to this problem.

These discoveries prompted a vast amount of research into penicillins, cephalosporins and their analogues, which continues to this day. The continuing interest in these compounds is understandable, as currently the world market in antibacterial agents is worth approx. six thousand million pounds, and roughly 65% of this is due to sales of β-lactam antibiotics. It is also interesting to note that β-lactams constitute 15% of the value of all drug sales.

This research effort has involved three areas of interest:

- -chemical modification of natural products,
- -screening of microbial metabolites for new antibacterial activity,
- -synthesis of new β-lactam-containing compounds.

Large numbers of modified natural products have been reported, including many examples where the C-6 (C-7) side chain has been altered. These have been covered by various review articles.[5-15]

It is the synthesis of new β-lactam derivatives which is relevant in the context of this thesis. Again, various reviews covering this area have been published.[9-16] The most recent comprehensive reviews in this area are those of Sammes[9] (1980), Morin and Gorman[15] (1982) and Durckheimer et al.[10] (1985). This introduction will provide an overview of the syntheses of β-lactam analogues containing a non-traditional ring system.

Syntheses of penicillin and cephalosporin analogues.

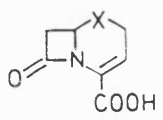
A considerable number of penicillin and cephalosporin analogues have been synthesised in which S-1 has been replaced by either a heteroatom, or by carbon; and/or in which C-2 or C-3 has been replaced by a heteroatom.

1.1 Cephalosporin analogues.

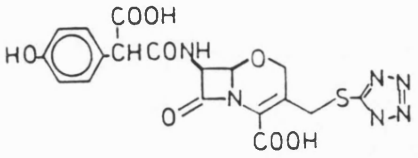
A wide range of cephalosporin analogues have been synthesised, including compounds containing two heteroatoms plus the bridgehead nitrogen in the 6-membered ring. The structures of cephalosporin analogues synthesised to date are summarised below.

There are many examples where the sulphur atom has been replaced by another heteroatom (1). The 1-oxacephems (1,X=O) were among the first such derivatives to be synthesised.[17] 1-oxacephems were found to be considerably more active than the parent cephems, as replacement of sulphur by oxygen in the cephem skeleton increases the ring strain, intensifies the pyramidal character of the β-lactam nitrogen and hence increases the reactivity of the βlactam bond. The molecule

is also found to be more hydrophilic. The 1-oxacephems have decreased β-lactamase stability with respect to the cephalosporins, but this can be overcome by introduction of a 7α-methoxy group and an oxime or carboxy function in C-2 of the 7-acetamido side chain. Some oxacephems are now available for clinical use; for example latamoxef (2), produced by Shionogi.

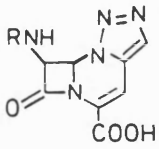


(1)



(2)

There are fewer 1-azacephem (1,X=NR) syntheses reported in the literature, and little biological data is available. However, in 1981 Pearson[18] reported the synthesis of triazolocephams (3) having antibacterial properties.

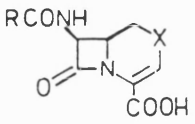


(3)

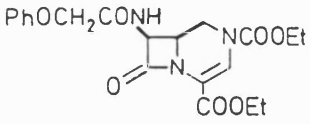
Another group of synthetic cephalosporin derivatives, the 1-carbacephems[19] (1,X=CH_2), is becoming increasingly important. Bristol Laboratories have been particularly active in this field, and have produced a number of active 1-carbacephems.

Examples of systems where sulphur has been replaced by a methylene group, and C-2 has been replaced by a heteroatom, have also been prepared. Examples of biologically active 1-dethia-2-oxacephems (4,X=O) and 1-dethia-2-thiacephems (4,X=S) have been reported. The 1-dethia-2-azacephems (4,X=NR) are vinylogous carbamic acids and therefore have

limited stability. However (5) does possess some modest antibacterial activity.

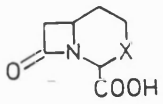


(4)



(5)

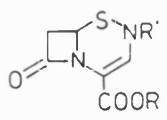
A number of other heterocyclic variants, less important in terms of biological activity, have been synthesised. The cepham derivatives in which S-1 has been replaced by a methylene group and a heteroatom has been substituted for C-3 have recently been reported (6). The examples synthesised displayed, at best, only modest antibacterial properties.



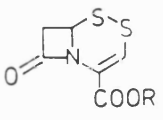
(6)

X=S 1-dethia-3-thiacephams
X=O 1-dethia-3-oxacephams
X=NR 3-aza-1-dethiacephams

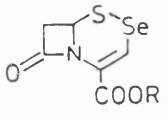
A variety of bicyclic derivatives containing two heteroatoms in the 6-ring (in addition to the bridgehead nitrogen) have also been synthesised. The structures of these compounds are shown below. It is interesting that the 2-aza-1-thiacephems (7), the 2-thiacephems (8) and the 2-selena-1-thiacephems (9) have all been used as intermediates in desulphurative routes to hetero-analogues of the penems. Little information has been published regarding the antibacterial activity of these compounds; in general these compounds seem to be either weakly active or antibacterially inactive.



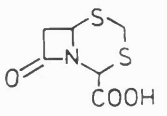
(7)



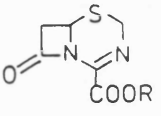
(8)



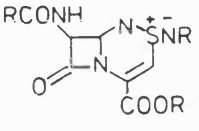
(9)



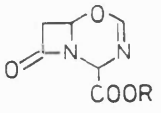
3-thiacephems



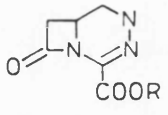
3-aza-1-thiacephems



1-aza-2-thia-(S-imido)-cephems

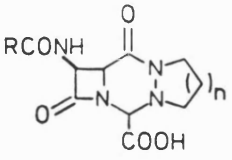


3-aza-1-oxacephems



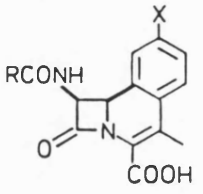
2-aza-3-aza-1-dethiacephems

In addition to the above bicyclic derivatives, some tricyclic derivatives have been reported by workers at both Beecham[18] (3) and Smith Kline and French[20] (10-12)



(10) n=1

(11) n=2

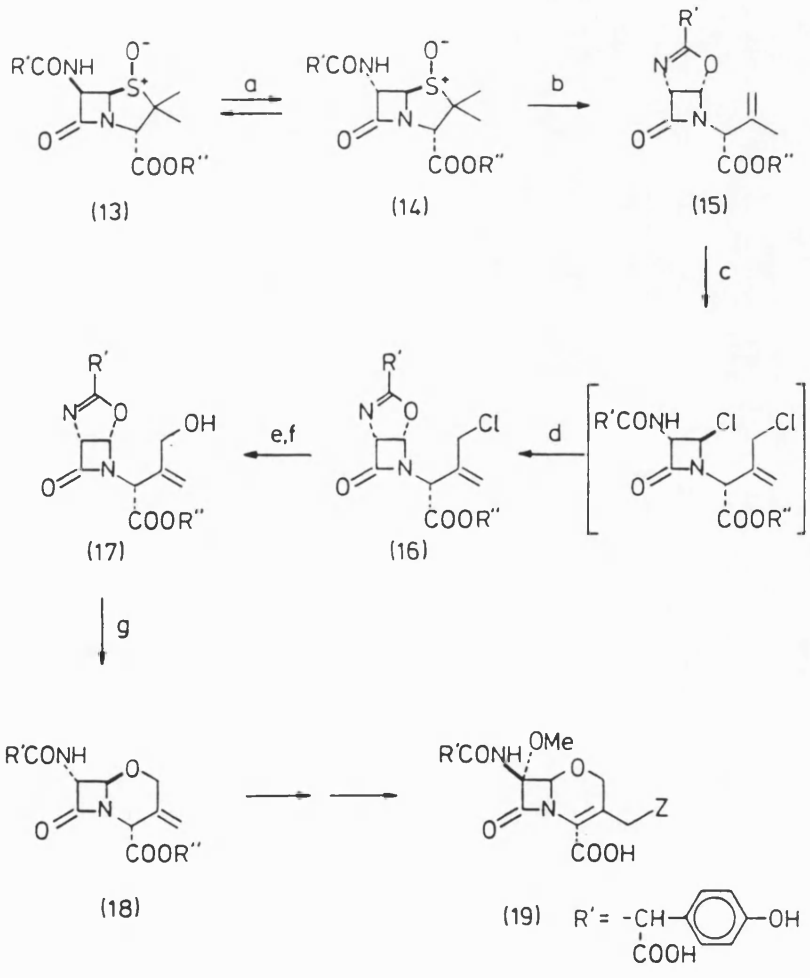


(12)

The syntheses of these hetero-analogues of the cephems will now be considered in detail.

1.1.1 1-oxacephems.

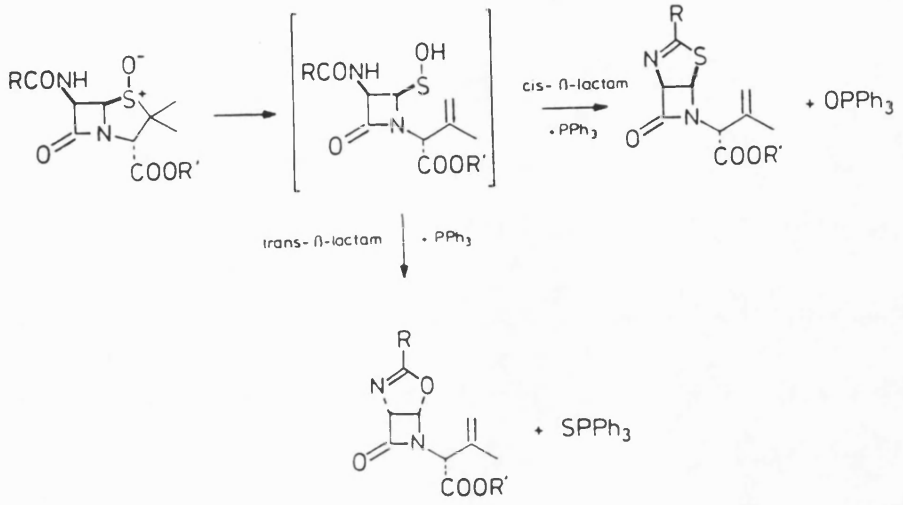
Stereoselective syntheses of the 1-oxacephems have been devised by several teams.[21] The Shionogi group have synthesised a range of 1-oxacephems, via opening of the thiazolidine ring of a penicillin sulphoxide, insertion of a reactive leaving group in the 4-position, and subsequent displacement of this leaving group by an oxygen nucleophile, effecting cyclisation.[22] In order to obtain the desired (R)-configuration at C-6, it is necessary to epimerise the adjacent chiral centre at C-7, which directs the incoming substituent into the **trans**- position. One such example is the synthesis of latamoxef (19)[23] (Scheme 1).



SCHEME 1.

a) base: b) PPh_3, 80°C: c) Cl_2: d) $NaHCO_3$: e) NaI: f) DMSO /H_2O, CuO: g) $BF_3.Et_2O$.

The penicillin S-oxide (13) can easily be epimerised with base to give (14). Opening of the thiazolidine ring by heating with triphenylphosphine results in the bicyclic compounds[24] (15). A similar ring opening of penicillin-S-oxides with normal configuration at C-6 has been described by Cooper et al.[25] (Scheme 2).



SCHEME 2.

Although direct allylic oxidation of (15) to (17) was intensively investigated, the indirect route via allylic chlorination with Cl_2 or SO_2Cl_2 to give (16), (which proceeds via an ene mechanism), proved to be superior for large scale synthesis.

Direct chlorine/hydroxy exchange is difficult due to the low reactivity of the allylic chloride (16). It is best to substitute iodide for chloride and to introduce the allylic -OH group with Cu_2O in DMSO/H_2O. It is assumed that DMSO forms an active alkylsulphonium intermediate with the allylic iodide. Cu_2O both activates the iodide and traps the resulting hydrogen iodide.

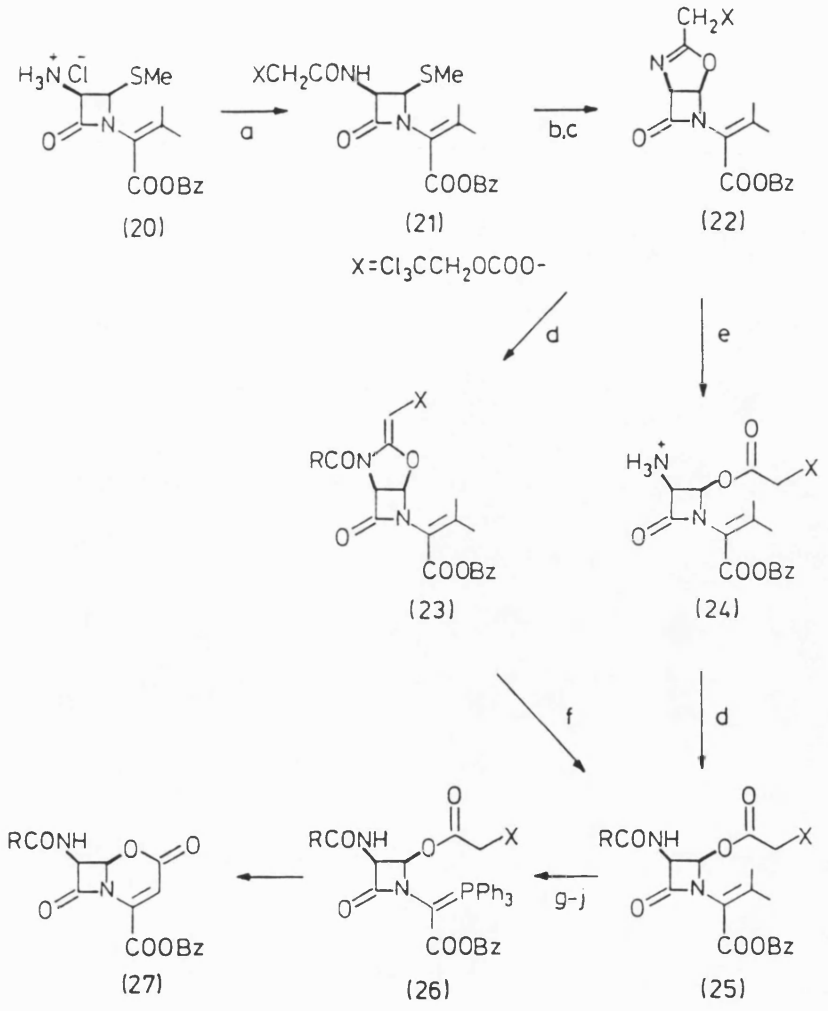
Allylic alcohol (17) is stereospecifically cyclised to (6R)-3-methylene-1-oxacepham (18) by proton or Lewis acid catalysis. This intermediate allows extensive variation at the 3-methylene group. Ozonolysis leads to 3-substituted oxacephems. Light induced addition of chlorine to (18) and elimination of HCl produces a 3-chloromethyl side chain which can react with a large number of nucleophiles.

The C-7 acylamido group can be brought back into the desired β-position by methoxylation. Unlike the cephalosporins, the 7β-amino-7α-methoxy oxacephems (19) do not epimerise in acidic medium, as the configuration of the amino group is stabilised by a strong hydrogen bond to the ring oxygen.[26]

The stereochemistry at C-6 and C-7 can be controlled by a different method from that shown in Schemes 1 and 2. If an oxygen nucleophile is passed on intramolecularly from the 3-amido side chain of the azetidinone by ring closure at C-4; only the **cis**- product is formed for steric reasons. This strategy was used by both Fujisawa[27] and Shionogi;[28] the synthesis of the 2-oxo-1-oxacephem (27), outlined in Scheme 3, is that of Fujisawa.

The azetidinone (20), produced by cleavage of 6-APA benzyl ester, is acylated to (21) with protected glycolic acid. The methylthio group can be cleaved off with elemental chlorine to yield a 4α-chloroazetidinone which cyclises with silver salt catalysis to oxazoline (22). Acylation at the dihydrooxazole nitrogen gives (23), and acid hydrolysis of the oxazolidine yields (25). The reaction sequence can also be reversed (22-24-25).

The oxazine ring is built up by an intramolecular Wittig reaction, ie the Woodward procedure;[29] ozonolysis of (25), followed by reduction of the resulting oxalic acid monoamide, then chlorination with thionyl chloride, and reaction with triphenylphosphine leads to the phosphorus



SCHEME 3.

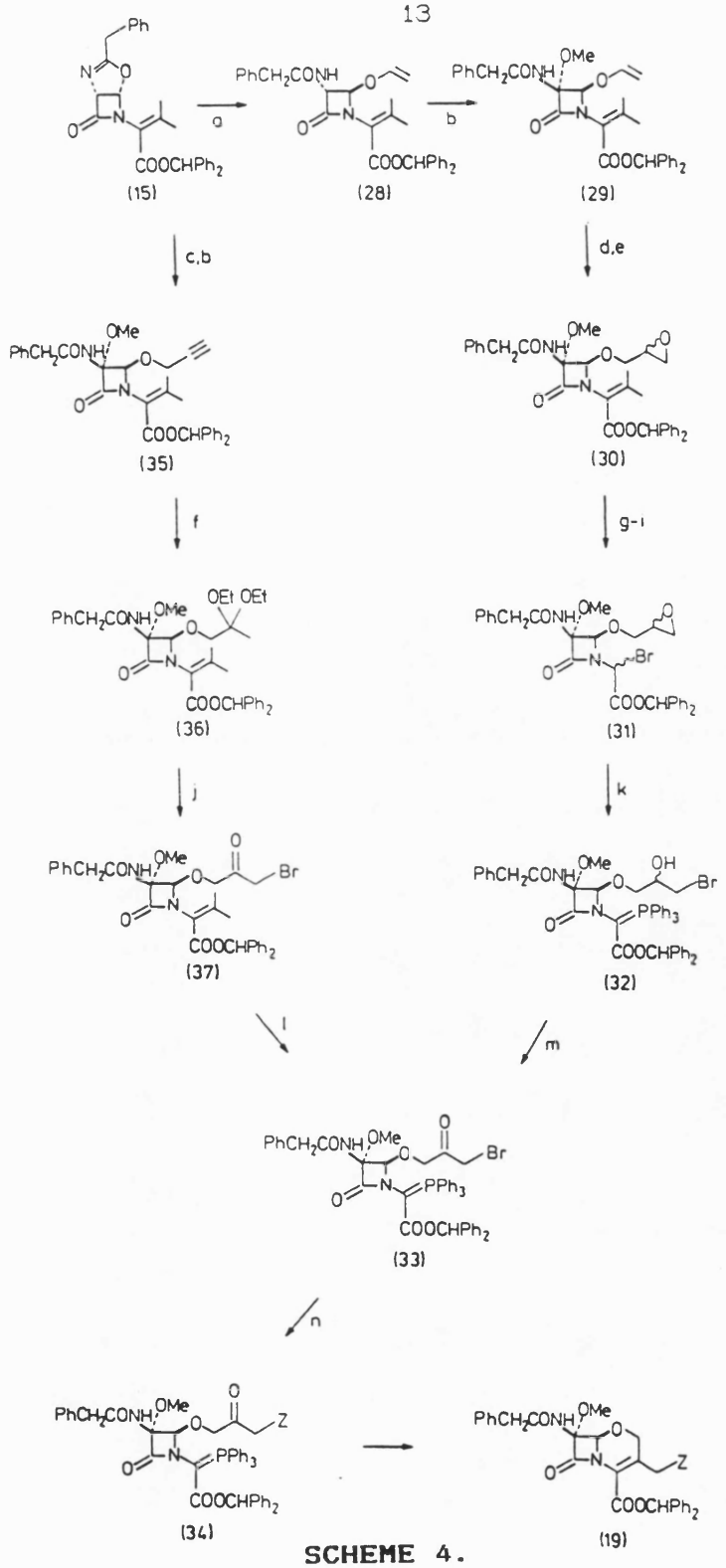
a) $Cl_3CCH_2OCOOCH_2COOH$: b) Cl_2: c) $AgBF_4$, Ag_2O: d) $PhOCH_2COCl$: e) camphorsulphonic acid, H_2O: f) p-TsOH, H_2O: g) O_3: h) Zn, EtCOOH: i) $SOCl_2$/py: j) PPh_3: k) Zn, AcOH: l) DMSO, Ac_2O.

ylid (26). The protecting group in (26) is cleaved off with zinc and glacial acetic acid, and the hydroxy ketone is oxidised by the Moffat method to the aldehyde, which cyclises spontaneously to the 2-oxo-1-oxacephem (27). In accordance with the instability of 2-oxocephalosporins reported by Ernest,[30] 2-oxo-1-oxacephems (27) were found to be very labile, and devoid of antibacterial activity.

The above route utilises an intramolecular Wittig reaction for construction of the bicyclic ring system. The Wittig cyclisation has been widely used in the synthesis of 1-oxacephems;[31] another example is due to the Shionogi group,[32] (Scheme 4) in which stereoselectivity is achieved by a similar strategy to that seen in a previous example (see Scheme 1).

Penicillin G was converted into the oxazolinoazetidinone (15), then cleaved stereospecifically with allyl alcohol to give the **trans** acetal (28) in high yield. Methoxylation at C-3 proceeded with inversion, giving (29): epoxidation was achieved by successive treatment with N-bromosuccinimide then t-butoxide. Conversion of the epoxide (30) into the bromoketone (33) could be achieved in one of three ways:[33] for example, bromination of the epoxide (30) gives (31); which, when treated with triphenylphosphine causes cleavage of the epoxide ring by the eliminated hydrogen bromide, yielding the bromohydrin (32). This can be converted into the bromoketone (33) by oxidation with chromium trioxide. Displacement of bromide by the desired group Z^- gave (34), which was then cyclised to the 1-oxacephem (19).

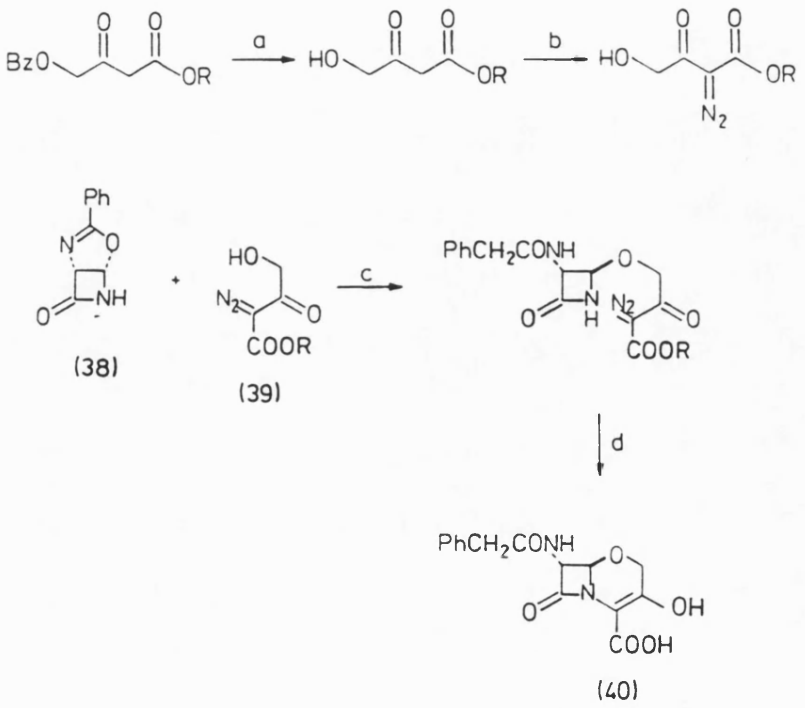
Alternatively, propargyl acetal (35) derived similarly from (15) was reacted with ethyl orthoformate and mercuric oxide in ethanol to give ketal (36). Bromination gave (37), which was converted into (33) via the standard sequence.



SCHEME 4.

a) allyl alcohol, CF_3SO_2H: b) Me_3COCl, LiOMe: c) propargyl alcohol, CF_3SO_2H: d) NBS, $DMSO/H_2O$: e) ^tBuOK: f) ethyl orthoformate, EtOH, HgO: g) O_3: h) Zn, AcOH: i) $SOBr_2$: j) $CuBr_2$, ethyl orthoformate, EtOH: k) PPh_3: l) standard procedure: m) CrO_3: n) NaZ.

In addition to the syntheses already described, in which the oxazine ring has been constructed via C-6 to O-1 or C-3 to C-4 cyclisation respectively, synthesis via C-4 to N-5 bond closure has also been achieved.[34] This approach involves etherification of the N-unsubstituted oxazoline-azetidinone (38) with the building block (39) and subsequent ring closure by Merck-type carbene insertion[35] (Scheme 5). Further conversion of the 7α-acetamido-1-oxacephem (40) into a 7β-derivative can be effected by various methods,[36] including that outlined previously.



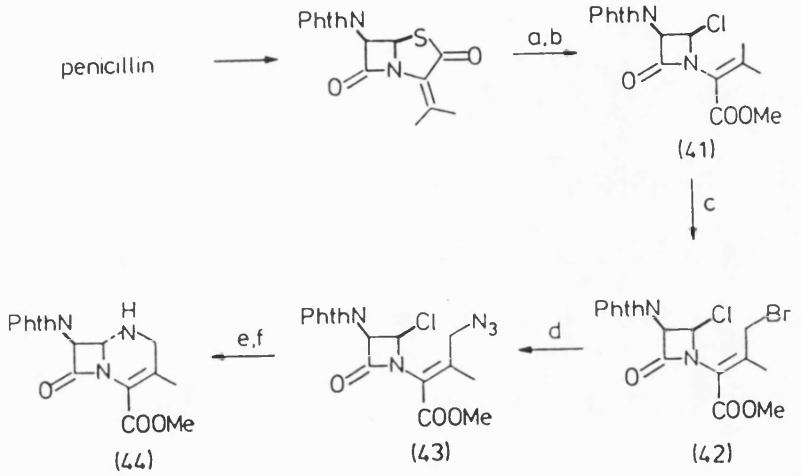
SCHEME 5.

a) H_2, Pd-C: b) p-TsN_3: c) $BF_3.Et_2O$: d) $Rh(OAc)_4$, benzene.

1.1.2 1-azacephems.

In 1972 Wolfe **et al.** reported the synthesis of 6-epi-azacephem[37] (44) starting from anhydropenicillin. (Scheme 6). Anhydropenicillin[38] was chlorinated to give a

chloroacid chloride; which, upon treatment with methanol, was converted to an epimeric mixture of chlorides (41). The epimers were separated and the 4-β epimer was brominated with N-bromosuccinimide, using AIBN as initiator. The resulting bromide (42) was treated with azide, giving (43), and hydrogenation of the azide group then treatment with potassium t-butoxide gave the 6-epiazacephem (44). No biological data was given, but it is unlikely that (44) would be active, as the **cis**-form is required for activity.

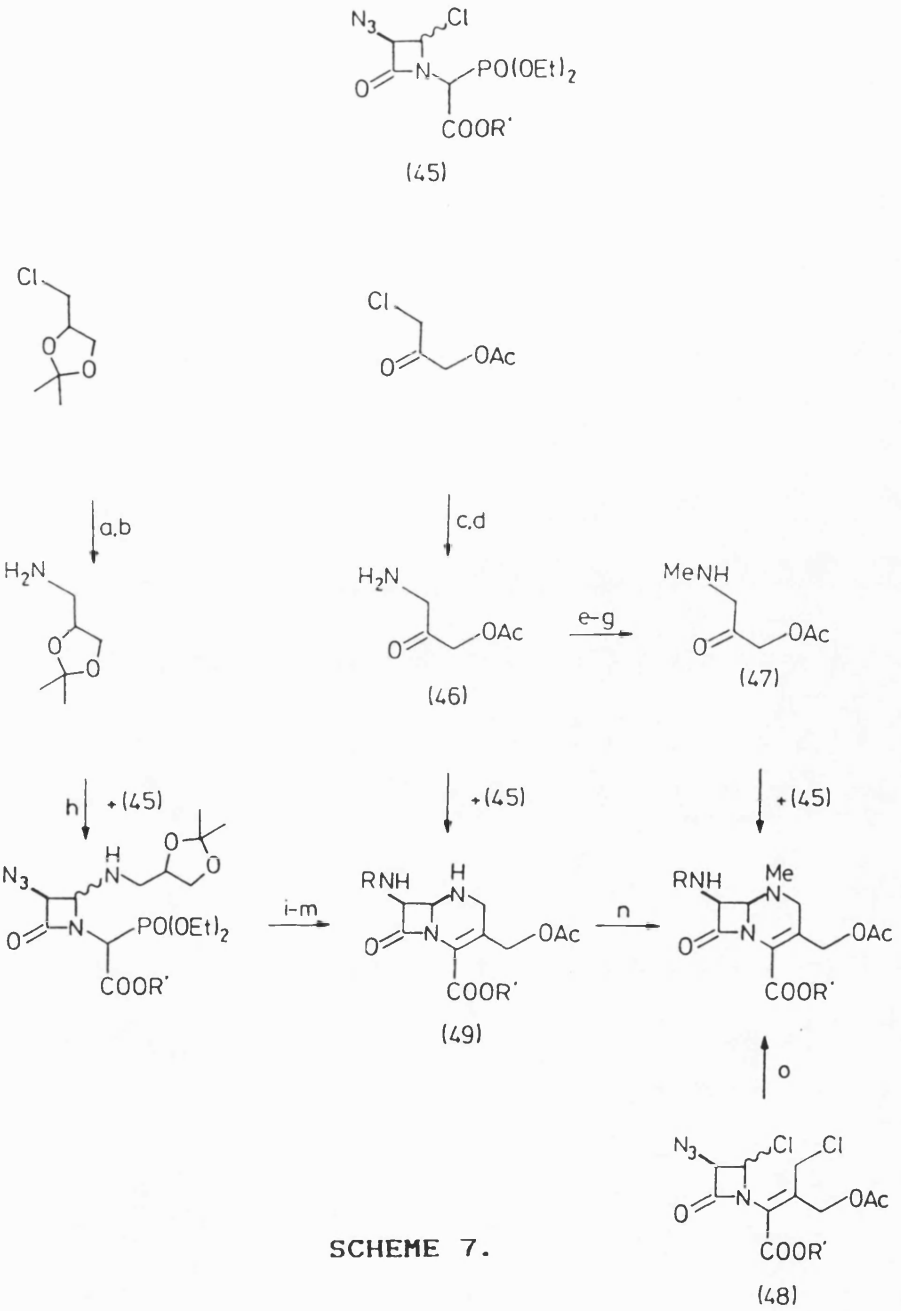


SCHEME 6.

a) Cl_2: b) MeOH: c) NBS, AIBN: d) NaN_3, DMF: e) H_2, PtO_2: f) ^tBuOH, ^tBuOK.

Wolfe later published a similar route to 1-oxacephems, also involving the intermediate bromide (42).

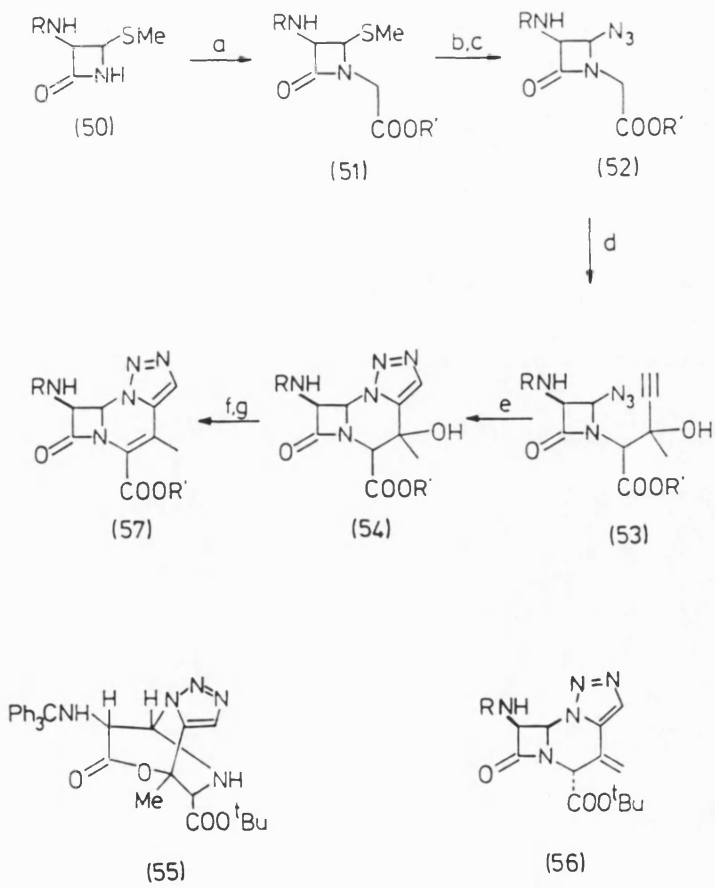
A Merck patent has described the synthesis of several racemic 1-azacephems.[39] These methods are outlined in Scheme 7. The 4-chloroazetidinone (45) is treated with a suitable 1-aminopropan-2-one derivative such as (46) or (47). The reaction between the dichloride (48) and an amine has also been used. The resulting 1-azacephems (49) have to be separated from the corresponding 7α-isomers.



SCHEME 7.

a) NaN_3, DMF: b) H_2, Pt_2O, benzene: c) NaN_3, THF, H_2O: d) H_2, Pd/C, EtOAc: e) PhCHO: f) Me_2SO_4, benzene, reflux: g) NaOH; HCl: h) ^tBuOH, ^tBuOK: i) py, $ClCO_2Bz$: j) 7% aq. $HClO_4$: k) py, Ac_2O: l) CrO_3, py: m) NaH, DMF, then usual sequence: n) HCHO, PtO_2, H_2, AcOH/H_2O: o) $MeNH_2$, DMF, K_2CO_3, then usual sequence.

Pearson et al. have reported some tricyclic analogues of the 1-azacephems.[40] (Scheme 8): the synthetic methodology used is similar to that used in the synthesis of tricyclic 2-aza-1-dethiapenams and penems, as the key step involves a 1,3-dipolar cycloaddition.



SCHEME 8.

a) $BrCH_2CO_2Bz$, K_2CO_3, DMF: b) Cl_2, CCl_4: c) NaN_3, DMF: d) $Li(SiMe_3)_2$, THF, but-3-yn-2-one: e) $PhCH_3$, reflux: f) $SOCl_2$, 2,6-lutidine: g) DBU.

Treatment of 4-methylthioazetidinone (50) with benzyl bromoacetate gave the ester (51), which was converted into the azide (52) via the chloride. Reaction of the ester enolate of (52), generated with $LiN(SiMe_3)_2$, with but-3-yn-2-one gave the alcohol (53) as a mixture of isomers. Cyclisation to (54) was effected in toluene under reflux. Chromatography on silica gel yielded starting material (53) (10%), the triazolocephem (54) as a mixture of isomers (57%) and another product (25%) which was identified as the lactone (55). Furthermore, recovered starting material (53) was a single isomer. When the latter was heated in toluene under reflux, lactone (55) was the sole product. It appears that one of the major isomers of the azide (52) gives a cycloaddition product which undergoes immediate thermal rearrangement to the lactone (55). Recrystallisation of (54) gave a single, crystalline isomer (85%). NMR data indicated that this has the ester group in the 5α-configuration; and as this isomer did not form a lactone, it was inferred that the 4-hydroxy group must have the α-configuration.

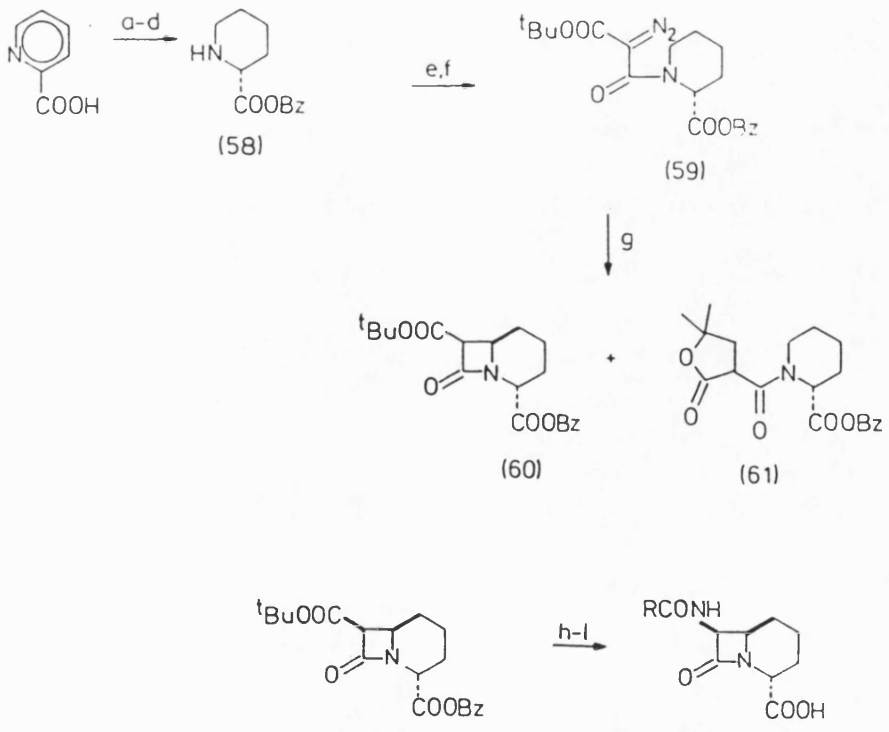
Treatment of the triazolocepham (54) with thionyl chloride/ lutidine gave a mixture of the corresponding chloride and the exomethylene compound (56), each compound being a single C-5 epimer. If (56) is not required, the crude mixture can be treated with DBU giving (57) in excellent overall yield.

1.1.3 1-carbacephems.

A considerable number of syntheses of 1-carbacephems have been reported,[41] including several approaches to compounds lacking a 4α-carboxylic acid function.[42,16] However, only those syntheses leading to 1-carbacephems having a carboxylic acid function will be discussed here.

The Oxford group has described the conversion of α-picolinic acid to the 1-carbacephem analogue[43] (185). (Scheme 9). The piperidine (58) was prepared from

α-picoline by catalytic reduction, resolution, and ester formation. Amide formation with the half-ester of malonic acid followed by diazoexchange gave (59). The photolysis of the diazo-amide gives an intermediate carbene which inserts into the piperidine ring to form a mixture of β-lactam products (60). The ɣ-lactone (61) is also produced, by carbene insertion into a C-H bond of the t-butyl group. Although the reaction is non-stereospecific with respect to the 6,7-substituents, a **trans**-relationship between C-4 and C-6 was obtained exclusively.

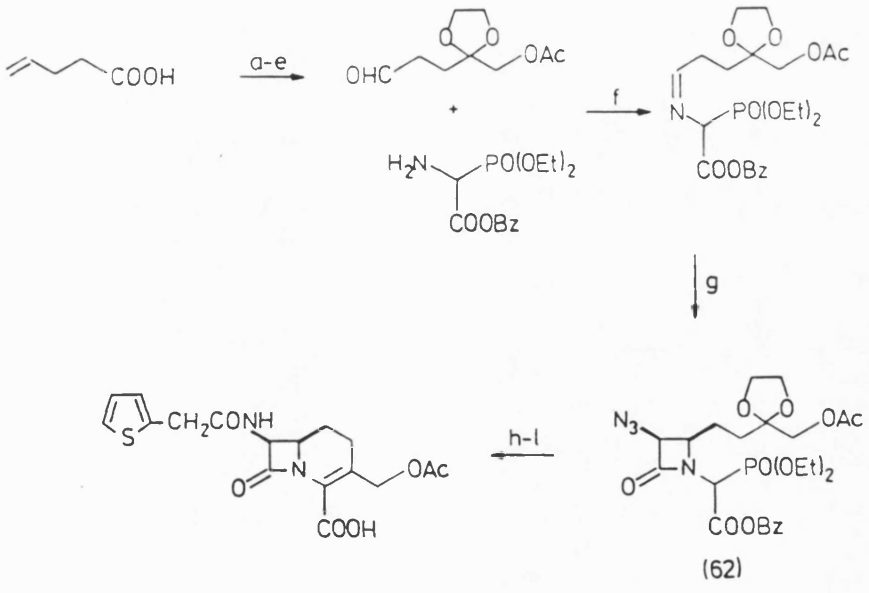


SCHEME 9.

a) catalytic hydrogenation: b) $ClCO_2Bz$: c) L-tyrosine hydrazide: d) benzylation: e) $^tBuO_2CCH_2CO_2H$, DCCI: f) p-TsN_3, Et_3N: g) hν: h) TFA: i) ^{t}butyl carbazate, DCCI: j) TFA, $NaNO_2$, HCl, Δ, ^tBuOH; then TFA: k) $PhCH_2COCl$, Et_3N: l) H_2, Pd.

Another important approach is that of the Merck team.[44] (Scheme 10). This involves the intermediate phosphonate (62), and complements their 1-oxa and 1-azacephem syntheses. A similar route to the 1α-hydroxy-1-carbacephems has been reported by Colvin et al. [45]

The key intermediate (62) is synthesised via the acetyl chloride-imine method, although other approaches to similar azetidinone intermediates have been described in a patent.[46]

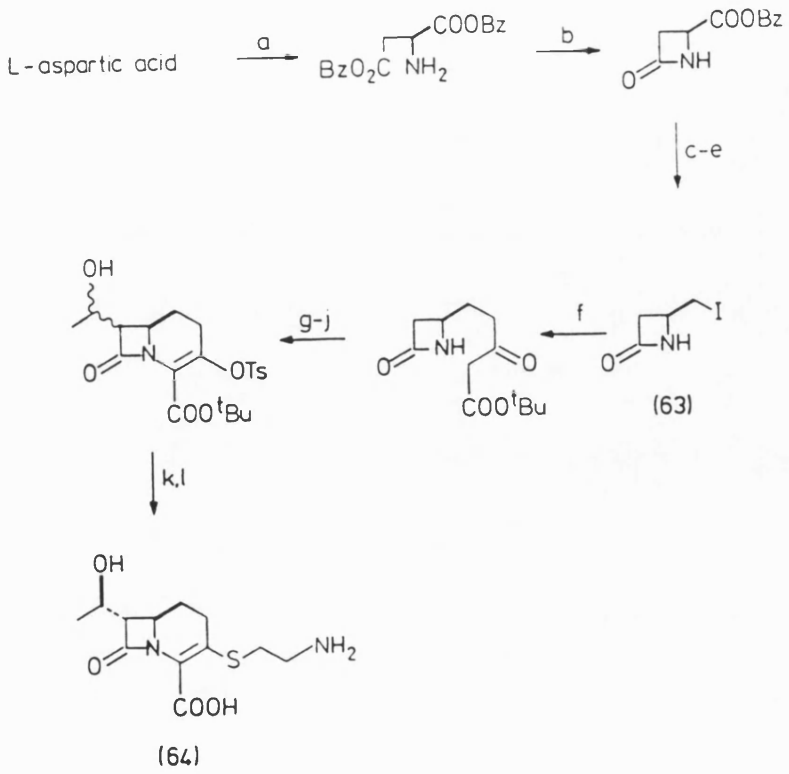


SCHEME 10.

a) $(COCl)_2$: b) CH_2N_2: c) AcOH: d) $HOCH_2CH_2OH$, p-TsOH, benzene, Δ: e) OsO_4, Et_2O, H_2O, $NaIO_4$: f) $MgSO_4$: g) N_3CH_2COCl, Et_3N, Et_2O: h) 10% aq. H_2SO_4, AcOH: i) py, AcCl: j) NaH, DME: k) 10% Pd/C, H_2, dioxane, H_2O: l) 2-thienylacetyl chloride, $NaHCO_3$.

Merck have also synthesised the carbacephem (64) in 12 steps from L-aspartic acid. This compound was potentially interesting, as it is the 4,6-ring homologue of thienamycin. The synthesis is outlined in Scheme 11. Surprisingly, if the

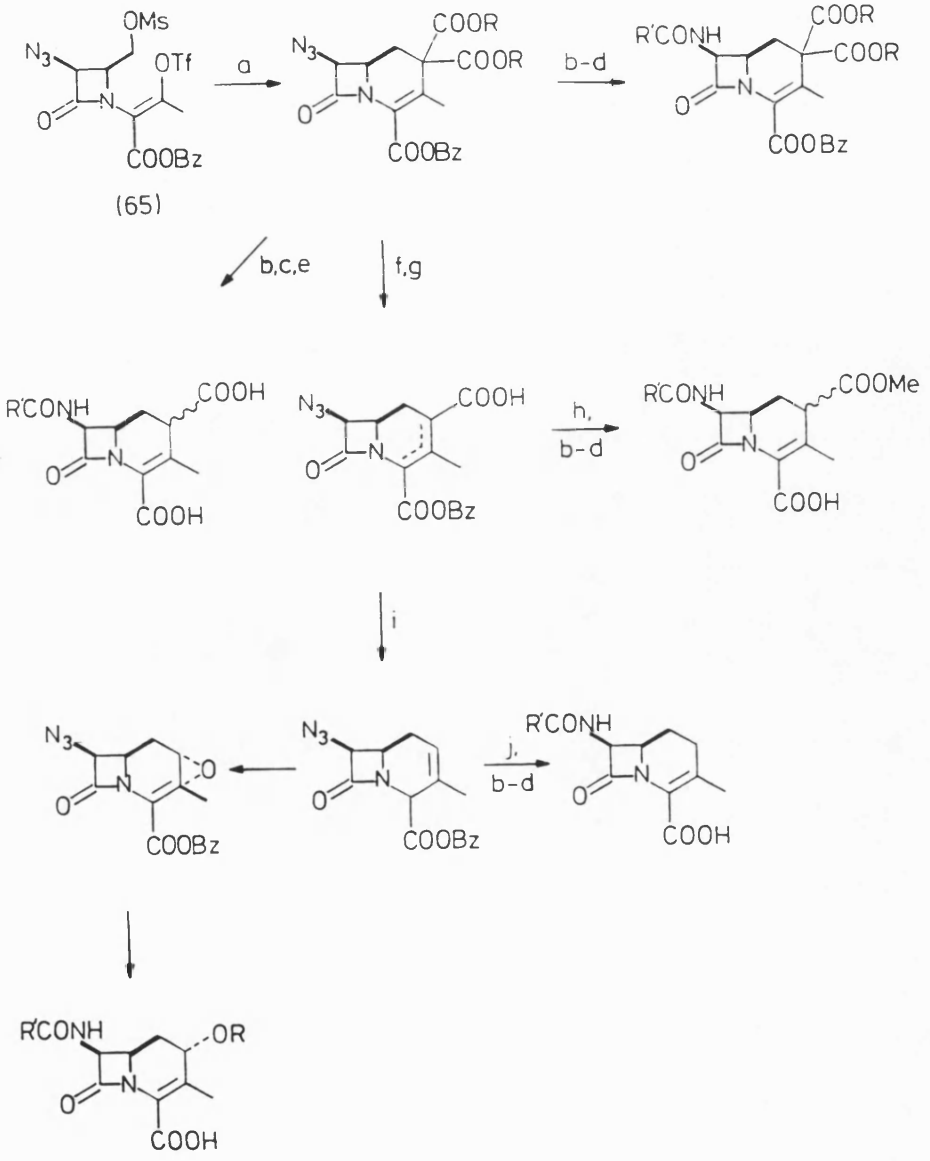
β-lactam (63) is protected by silylation, there is no reaction with the lithium dienolate of t-butyl acetate. The displacement of iodide is achieved by use of 2 moles of the dienolate on the unprotected azetidinone (63). In the reaction of the 7-carbanion with acetaldehyde, the required isomer is formed in 36% yield. It was found that (64) is much more chemically stable than thienamycin, but also shows greatly reduced biological activity.



SCHEME 11.

a) benzylation: b) TMSCl, Et_3N: c) $NaBH_4$: d) MsCl: e) NaI:
f) dienolate of $CH_3COCH_2CO_2{}^tBu$: g) $HO_2CC_6H_4SO_2N_3$:
h) $Rh_2(OAc)_4$: i) Ts_2O: j) LDA, CH_3CHO: k) $HSCH_2CH_2NH_2$:
l) TFA, anisole, Dowex 50.

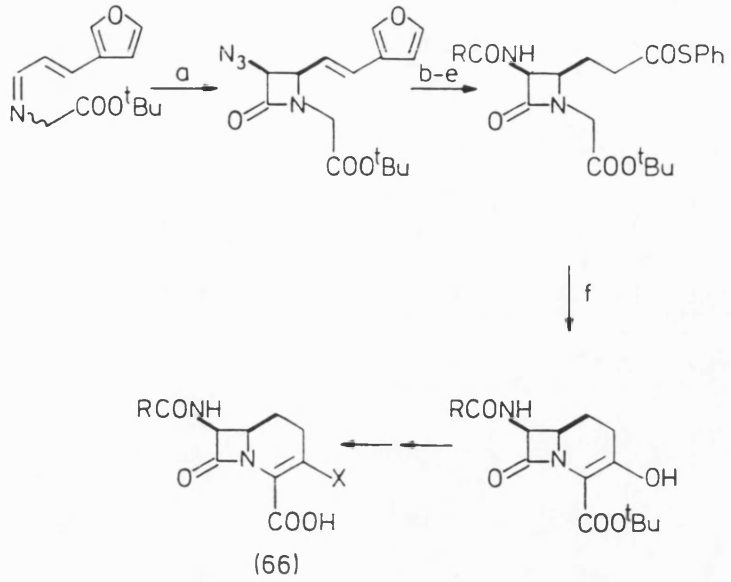
Bristol Laboratories have described the total synthesis of a variety of 2-substituted 1-carbacephems.[47] involving the important substituted azetidinone (65). (Scheme 12). This intermediate is made in 8 steps from a β-keto ester.



SCHEME 12.

a) $Na^+ {}^-CH(CO_2CH_2R)_2$, THF: b) H_2S, Et_3N: c) $PhOCH_2CO_2H$, EDDQ: d) H_2, Pd/C: e) H_2, Pd/C, AcOH: f)TFA, 60°C: g) toluene, Δ: h) $ClCO_2Me$, Et_3N: i) Δ, Et_3N: j) DBN.

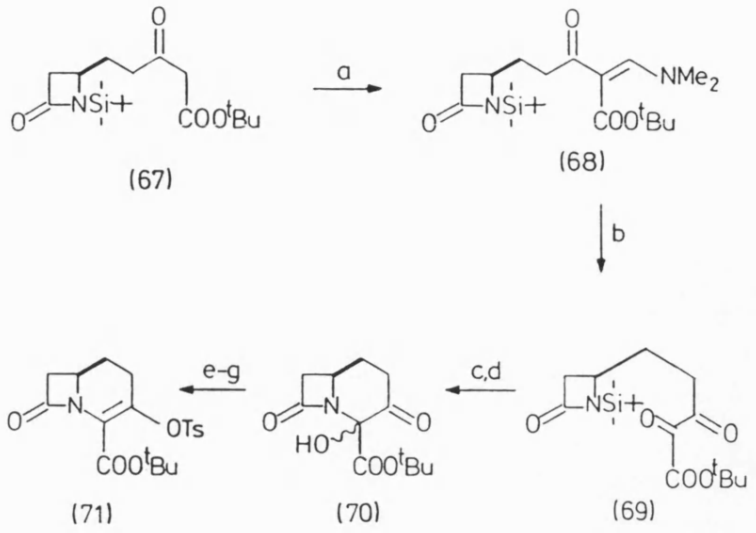
A recent synthesis of 3-heterosubstituted 1-carbacephems involves Dieckmann condensation as a key step.[48] This approach is outlined in Scheme 13. The 1-carbacephem (66, $X=SCH_2CH_2NH_2$) had considerable activity towards gram positive organisms.



SCHEME 13.

a) azidoacetyl chloride, Et_3N, CH_2Cl_2: b) H_2, $Pd-SrCO_3$: c) $PhOCH_2Cl$: d) O_3: e) PhSH, DCC, CH_2Cl_2: f) $LiN(SiMe_3)_2$.

Another recent synthesis of 3-heterosubstituted 1-carbacephems involves the vicinal tricarbonyl intermediate (69)[49] (Scheme 14). The silylated chiral β-lactam derivative (67), prepared by a route analogous to a Merck procedure,[50] was treated with N,N-dimethylformamide dimethyl acetal, giving the enamino ketone (68). Oxidation of (68) with singlet oxygen in $CDCl_3$ gave (69). Desilylation then led to (70), and conversion to the 3-tosyl 1-carbacephem (71) was achieved by treatment with TMSI, aqueous work-up ($K_2S_2O_3$), then treatment with p-toluenesulphonyl anhydride.



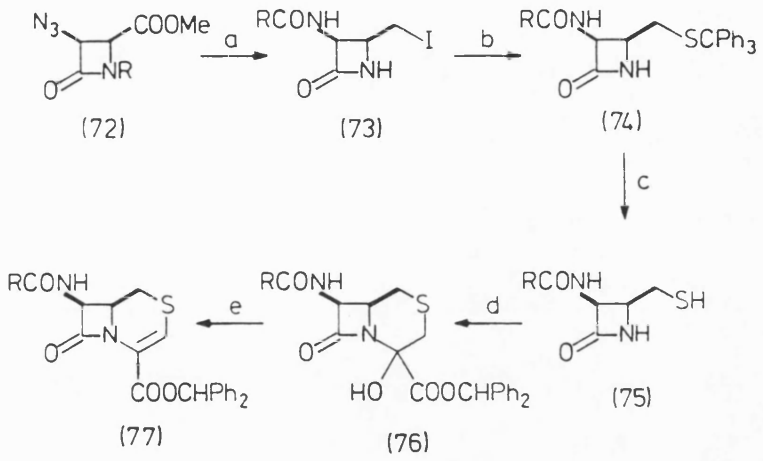
SCHEME 14.

a) $Me_2NCH(OMe)_2$: b) $^1O_2$, $CDCl_3$: c) HF/py, MeCN: d) CH_2Cl_2, mol. sieves: e) TMSI: f) $K_2S_2O_3$ aq.: g) p-tosyl anhydride, Et_3N.

1.1.4 2-heterosubstituted cephems.

Smith, Kline & French have published a total synthesis of 2-thiacephems[51] involving reaction of α-keto-β-bromoesters with the azetidinone (75). (Scheme 15). Synthesis of the useful azetidinone starting material (72) and its conversion to the 4-iodomethyl azetidinone (73) has been described elsewhere in this thesis.[52] Transformation of (73) to the corresponding thiol (75) was achieved by displacement of the iodide with the sodium salt of trityl mercaptan, and cleavage of the resulting thioether (74). Alkylation of thiol (75) with benzhydryl β-bromopyruvate

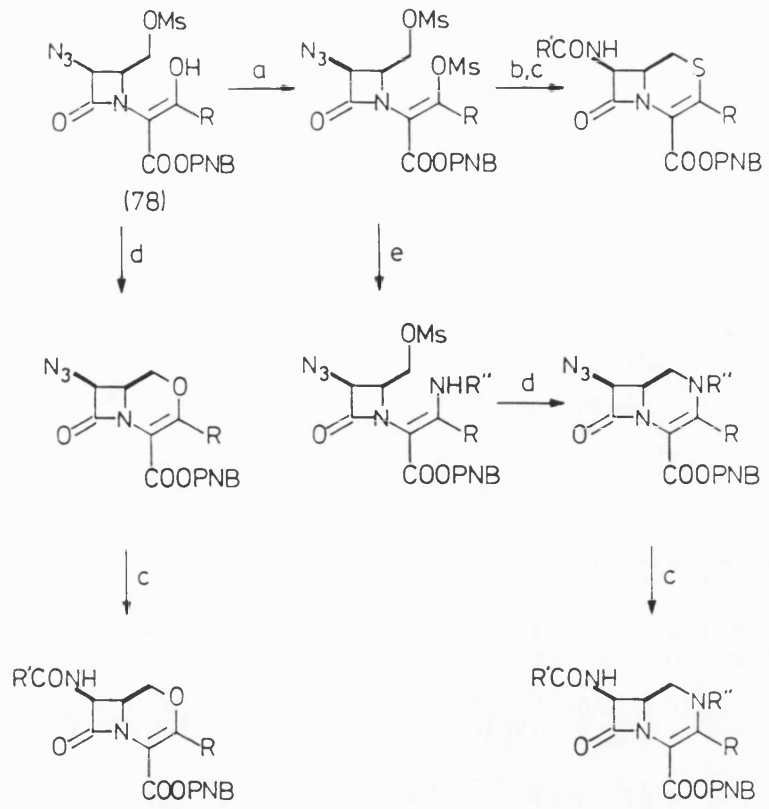
yielded the carbinolamide (76), which gave the 2-thiacephem (77) on dehydration with thionyl chloride/pyridine. The corresponding free acid was reported to be biologically active.



SCHEME 15

a) see Scheme 52: b) $Ph_3CS^-Na^+$: c) $AgNO_3$, MeOH:
d) benzhydryl β-bromopyruvate: e) $SOCl_2$, py.

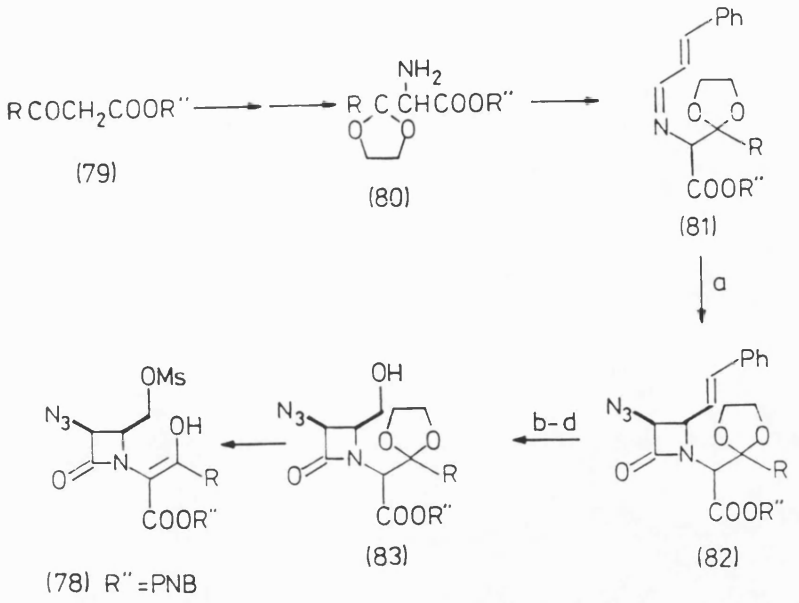
Bristol Laboratories have reported an alternative approach,[53] involving the key intermediate (78), which has also been used in syntheses of 2-oxacephems[54] and 2-azacephems.[55] The synthetic utility of this intermediate is demonstrated in Scheme 16. Tricyclic 2-oxacephem derivatives have also been synthesised using this methodology.[56]



SCHEME 16

a) MsCl, Et_3N, CH_2Cl_2: b) H_2S, Et_3N, CH_2Cl_2:
c) standard sequence: d) NaH, DMSO: e) RNH_2, DMSO.

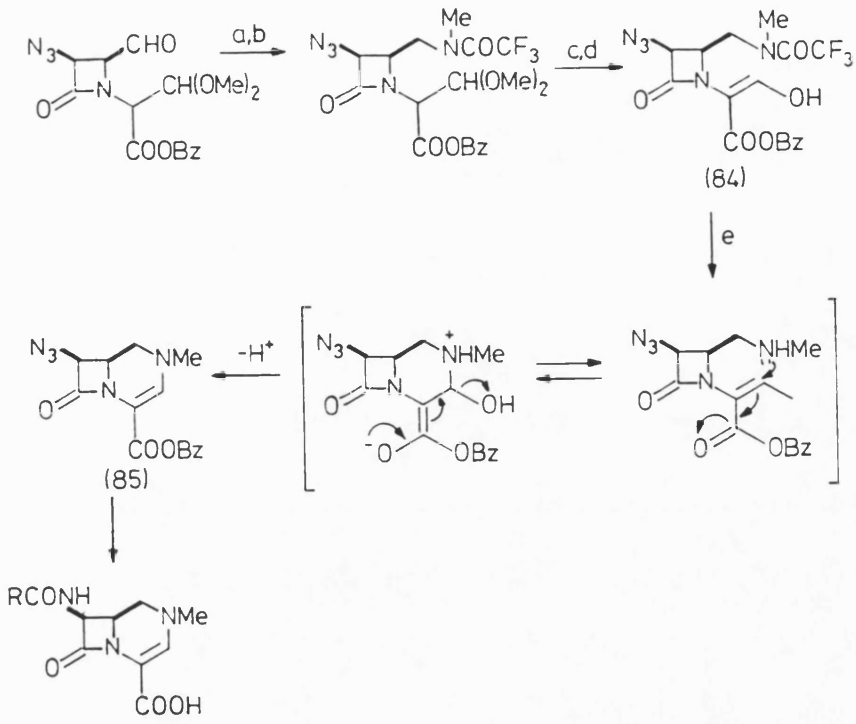
The synthesis of the intermediate (78) is outlined in Scheme 17. The β-ketoester (79) was converted via (80) into the Schiff's base (81). Reaction of Schiff's base (81) with azidoacetyl chloride yielded the functionalised azetidinone (82). The styryl group of (82) was converted into a hydroxymethyl group by ozonolysis; decomposition of the ozonide with dimethyl sulphide, then reduction with sodium borohydride gave (83). Conversion of (83) to (78) was achieved by mesylation of the alcohol moiety, and hydrolysis of the acetal group.



SCHEME 17.

a) N_3CH_2COCl, Et_3N: b) O_3: c) Me_2S: d) $NaBH_4$:
e) mesylation then acetal hydrolysis.

An alternative approach to the 2-azacephems is also reported.[57] This involves the N-trifluoro-acetyl protected derivative (84) as a key intermediate. Following deprotection of the trifluoroacetyl group with sodium borohydride, it is assumed that Michael-type intermolecular nucleophilic attack by nitrogen, followed by loss of hydroxide ion, leads to the 2-azacephem (85). (Scheme 18). However, the cyclisation step was found to proceed in poor yield, and the Bristol workers abandoned this approach in favour of that described earlier.

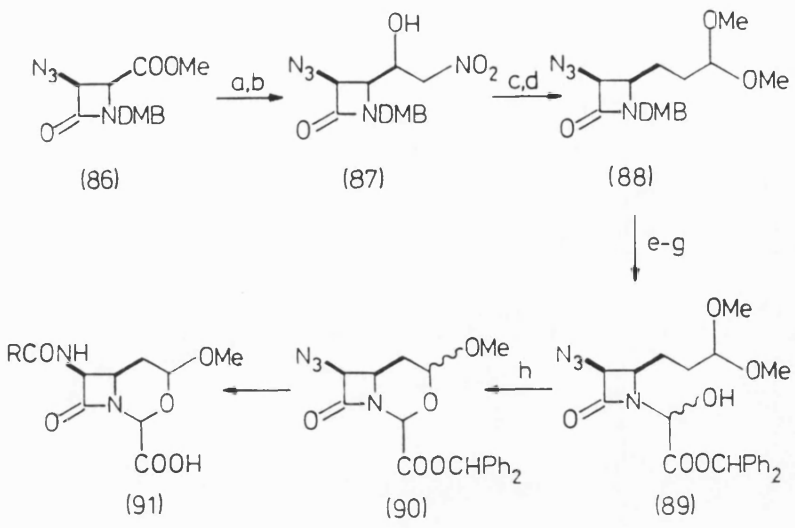


SCHEME 18.

a) $MeNH_2$, cyclohexane, $NaBH_4$, dioxane: b) $ZnCl_2$, TFAA: c) pyrrolidine, AcOH, benzene: d) aq. HCl, acetone: e) $NaBH_4$, EtOH, H_2O.

1.1.5 3-oxa-1-dethiacephams.

Recently the synthesis of 3-oxa-1-dethiacephams has been reported. One approach is due to the Smith, Kline & French group.[58] (Scheme 19).

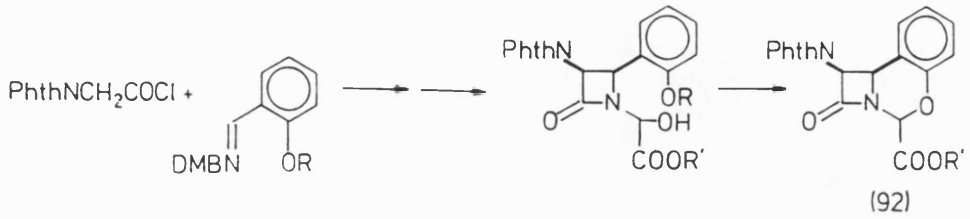


SCHEME 19.

a) $NaBH_4$, aq. THF: b) DMSO, TFAA, Et_3N: c) $MeNO_2$, Et_3N, DMSO: d) Ac_2O, py, $NaBH_4$, MeOH: e) NaOMe, H_2SO_4, MeOH: f) $K_2S_2O_8$, aq. MeCN: g) Ph_2CHO_2CCHO, toluene: h) TsOH, mol.sieves, CH_2Cl_2.

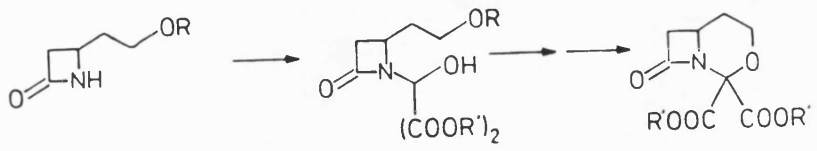
The azetidinone (86), prepared by cycloaddition of an azidoketene precursor to N-2,4-dimethoxybenzyliminoacetic ester, was selectively reduced to the alcohol, oxidised to the aldehyde, then condensed with nitromethane to give (87). Dehydration and subsequent reduction gave the saturated nitroethylazetidinone, which was transformed to the acetal (88) by a modified Nef reaction. Oxidative cleavage of the DMB protecting group, then condensation with benzhydryl glyoxylate gave (89) as a mixture of diastereomers, separable by chromatography. Cyclisation of (89) gave (90), which was converted into the 3-oxa-1-dethiacepham (91) using standard methodology.

Kametani et al. have reported the synthesis of 1,2-benzo -3-oxacephams (92), using similar methodology.[59] (Scheme 20).



SCHEME 20.

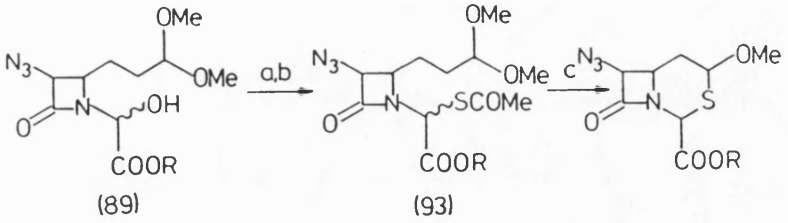
An alternative procedure involving a [1,2] anionic NOC⁻ to NCO⁻ rearrangement has recently been reported.[60] This is outlined in Scheme 49, and an analogous approach to 2-oxa-penams has been discussed elsewhere in this thesis.[61]



SCHEME 21.

1.1.6 3-thia-1-dethiacephams.

The Smith, Kline & French intermediate (89) has also been used in the synthesis of 3-thia-1-dethiacephams.[62] (Scheme 22). This intermediate was converted to the more reactive chloride, and reaction with potassium thioacetate gave the thioester (93). Cyclisation was effected under acid catalysis, and proceeded in 13% yield.

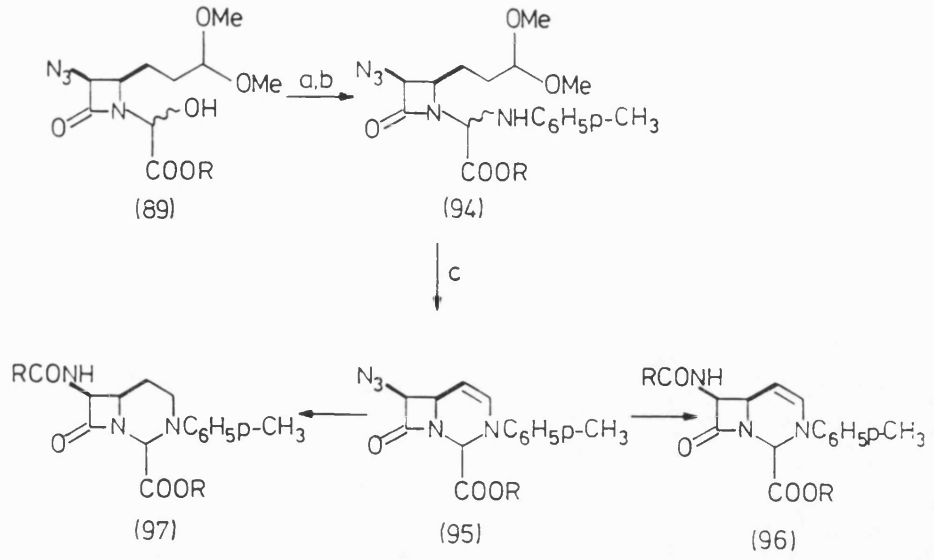


SCHEME 22.

a) $SOCl_2$, py: b) NaSCOMe: c) BF_3, MeOH.

1.1.7 3-aza-1-dethiacephems.

A similar approach was used in the preparation of the 3-aza analogue.[62] (Scheme 23). The intermediate (89) was converted into the chloride and treated with toluidene, giving a mixture of diastereomeric amines (94). One diastereomer cyclised smoothly under mild acid catalysis, giving the 3-aza-1-dethiacephem (95). This could be elaborated to the derivatives (96) and (97). (96) was found to possess weak antibacterial activity.

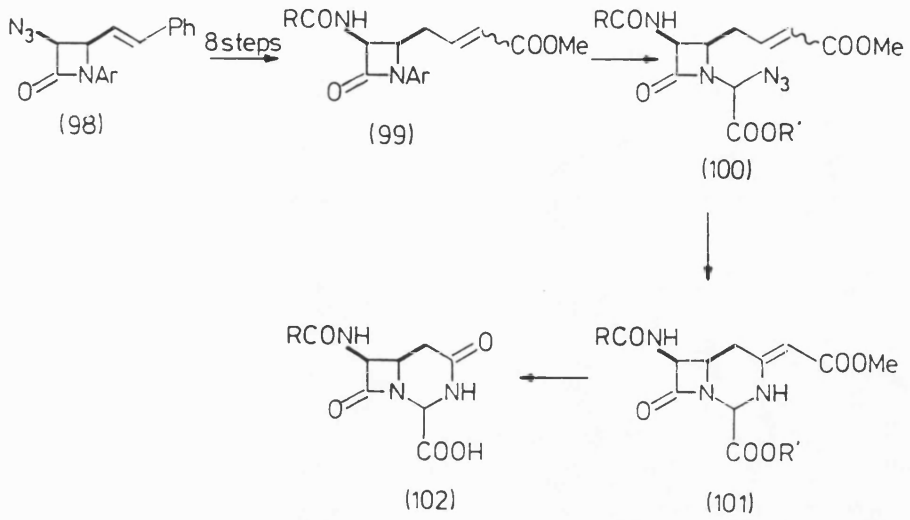


SCHEME 23

a) $SOCl_2$, py: b) toluidine: c) BF_3, MeOH.

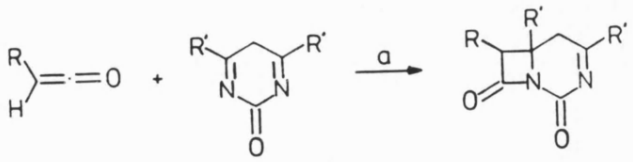
An alternative method involving 1,3-dipolar cycloaddition has been reported by the Beecham group.[63] (Scheme 24).

The azetidinone (98) was converted in an 8-step sequence into the azetidinone (99), obtained as a 1:2 mixture of **cis**- and **trans**- isomers in 40% overall yield. Removal of the N-protecting group, followed by the standard glyoxylate procedure, yielded the azide (100), which was cyclised in toluene under reflux. Initial 1,3-dipolar cycloaddition was followed by loss of nitrogen and tautomerisation to the enamine (101). Ozonolysis and deprotection of the 4-carbonyl group by catalytic hydrogenation gave (102), which was found to possess weak antibacterial activity.



SCHEME 24.

A recent paper[44] reports the synthesis of 4-oxo-3-aza-cephems via [2+2] cycloaddition. This is outlined in Scheme 25.

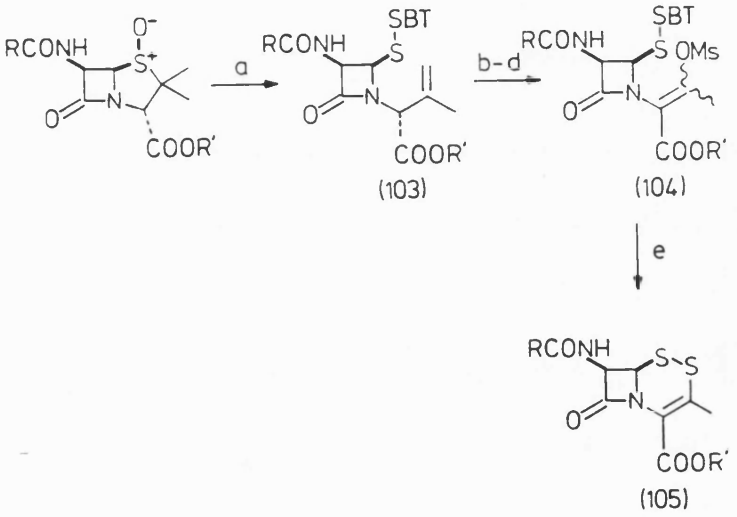


SCHEME 25.

a) Et_3N.

1.1.8 2-thiacephems.

The synthesis of 2-thiacephems and their subsequent conversion to penems has been reported by both Hoechst[65] and Farmitalia Carlo Erba.[66] (Scheme 26). The synthesis involves cleavage of the thiazolidine ring of a penicillin sulphoxide, giving (103); ozonolysis followed by mesylation gave the intermediate (104). Treatment of (104) with sodium hydrosulphide gave the 2-thiacephem (105).



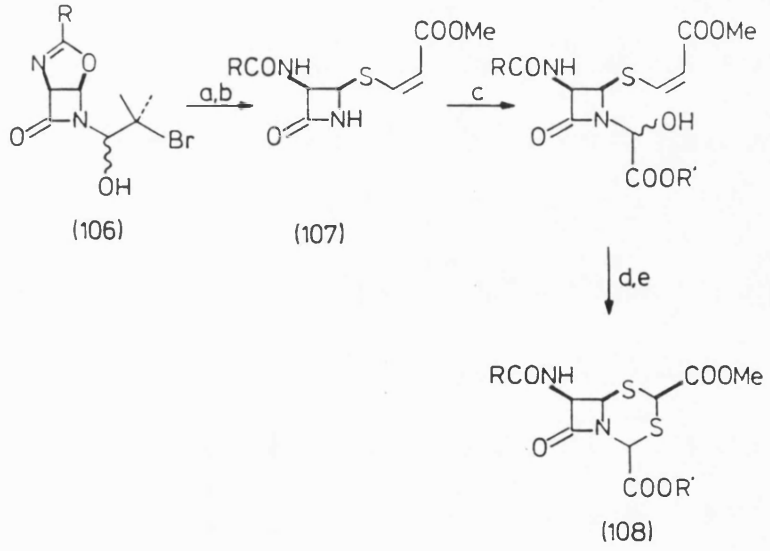
SCHEME 26.

a) HSBT, toluene, reflux: b) O_3: c) Me_2S: d) MsCl, py: e) NaSH, H_2O/EtOAc.

1.1.9 3-thiacephems.

A recent paper by Stoodley *et al.* describes a synthesis of 3-thiacephems.[67] (Scheme 27). The bromohydrin[68] (106) was treated with **cis**-β-(methoxycarboxyl)vinylisothiouronium chloride,[69] followed by treatment with triethylamine and

chromatography on silica, gave the azetidinone (107), together with its diastereomer. Following separation, sequential treatment of (107) with t-butyl glyoxylate, thionyl chloride/2,6-lutidine and hydrogen sulphide/ triethylamine, gave the 3-thiacephem (108). On the basis of nuclear Overhauser effect difference spectroscopy, it was shown that the 2- and 6- protons are **cis**- orientated, hence the 3-thia-cephem produced has the stereochemistry shown in structure (108).

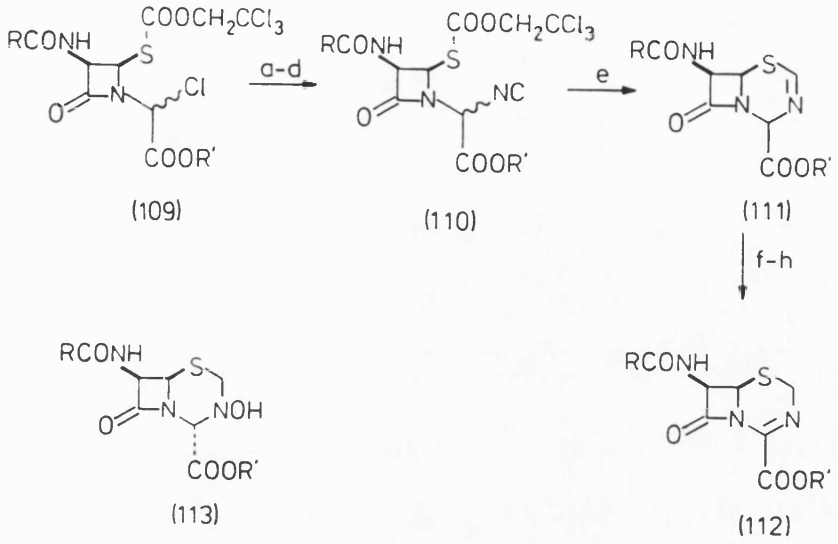


SCHEME 27.

a) **cis**-β-(methoxycarbonyl)vinylisothiouronium chloride, CH_2Cl_2: b) Et_3N: c) RO_2CCHO: d) $SOCl_2$, 2,6-lutidine: e) H_2S, Et_3N.

1.1.10 3-azacephems.

The Fujisawa group have reported a synthesis of 3-aza cephems[70] involving intramolecular cyclisation of the isocyanide (110) as a key step. (Scheme 28).

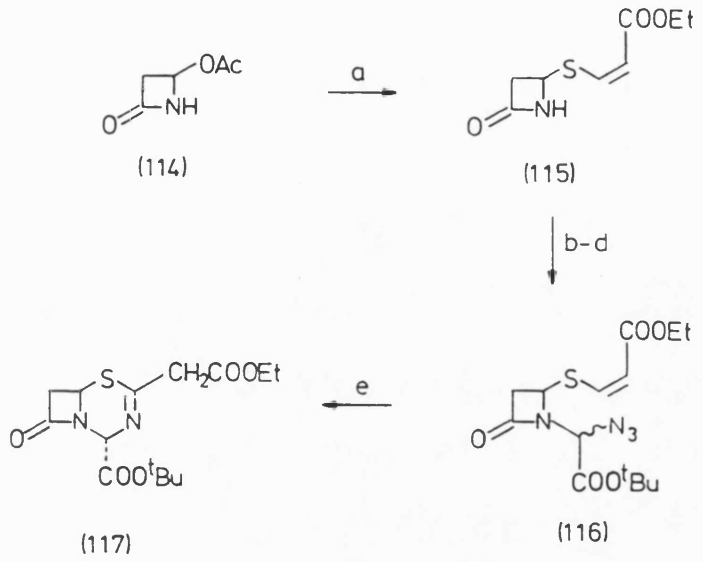


SCHEME 28.

a) NaN_3, DMF: b) HCO_2H, Pd/C: c) HCO_2H/Ac_2O: d) $POCl_3$, 2,6-lutidine, CH_2Cl_2: e) Zn dust, AcOH, DMF: f) Al/Hg, THF/H_2O: g) $(CF_3SO_2)_2O$, 2,6-lutidine, CH_2Cl_2: h) DBU, CH_2Cl_2.

The azetidinone (109) was synthesised from penicillin V using standard methodology. Conversion of (109) into (110) was achieved by treatment with sodium azide, reduction of the azide, then formylation and treatment of the resultant formamide with phosphorus oxychloride. Removal of the trichloroethoxycarbonyl protecting group produced an intermediate thiol which cyclised spontaneously to the Δ^2-3-azacephem (111). Conversion of (111) to the Δ^3-3-azacephem (112) was achieved by reduction of the C-2 to

N-3 double bond, acylation with triflic anhydride, then treatment with DBU, yielding the Δ^3-3-azacephem (112). This new bicyclic system was found to be very unstable, and attempts to remove the benzyl protecting group were unsuccessful. However, the deprotected Δ^2-3-azacephem (111, R'=H) and the compound (113) were found to possess antibacterial activity.



(114) (115) (116) (117)

SCHEME 29.

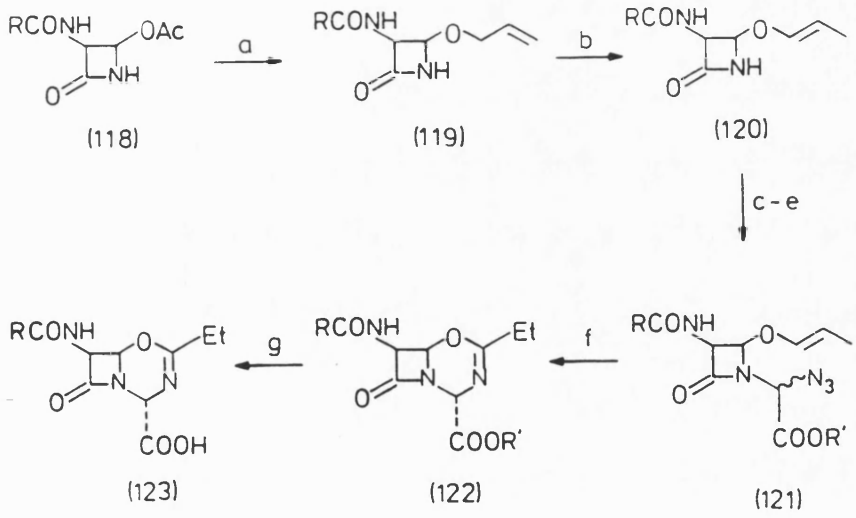
a) $NaSCH=CHCO_2Et$: b) RO_2CCHO: c) $SOCl_2$, py: d) NaN_3: e) xylene, reflux.

Recently the Beecham group have described an alternative method for the synthesis of 3-azacephems,[71] involving intramolecular cycloaddition. (Scheme 29). Reaction of 4-acetoxyazetidinone (114) with the sodium salt of ethyl β-mercaptoacrylate[69] gave the azetidinone (115), together with the **trans**-isomer. These were separated by crystallisation. Conversion of (115) into the azide (116) was accomplished via the standard procedure. Cyclisation was achieved under reflux in xylene, giving the 3-azacephem (117) as a single diastereomer. The recovered starting material was

found to be considerably enriched in one diastereomer: cyclisation of this diastereomer being less favourable due to steric interaction between the t-butoxycarbonyl group and the β-lactam carbonyl group.

1.1.11 3-aza-1-oxacephems.

The Beecham group have also described a synthesis of 3-aza-1-oxacephems[71] (Scheme 30) using a similar route to that described in the previous section. It was expected that cycloaddition would be more facile in this case.



SCHEME 30.

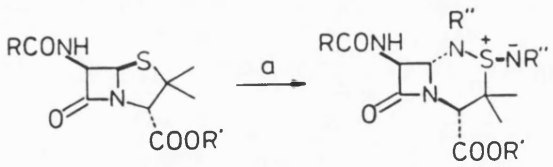
a) prop-2-enol, benzene, zinc acetate: b) 10% Pd/C, dioxane, reflux (Ar): c) $R'O_2CCHO$: d) $SOCl_2$, py: e) NaN_3: f) toluene, reflux: g) H_2, Pd/C.

The introduction of the desired vinyl ether functionality was achieved thus: the azetidinone (118)[72] was warmed with prop-2-enol in benzene containing zinc acetate, yielding a mixture of the **cis**- and **trans**- isomers of (119). These were readily separated by chromatography, and the **trans**-compound was isomerised to the desired **cis**- product.[73] Treatment with 10% Pd-C under argon in dry dioxane at reflux

gave the desired propenyl ether (120) as an inseparable mixture of **E** and **Z** isomers. Conversion of (120) into the azide (121) was carried out in the usual way, and cyclisation to (122) was achieved in toluene under reflux. The deprotected 1-oxa-3- azacephem (123) was devoid of antibacterial activity.

1.1.12 1-aza-2-thiacephams.

Campbell et al. have reported an example of these compounds,[74] synthesised via reaction of chloramine τ (sodium N-chlorotoluene-p-sulphonamide trihydrate) with 6β-substituted penicillanates. (Scheme 31).

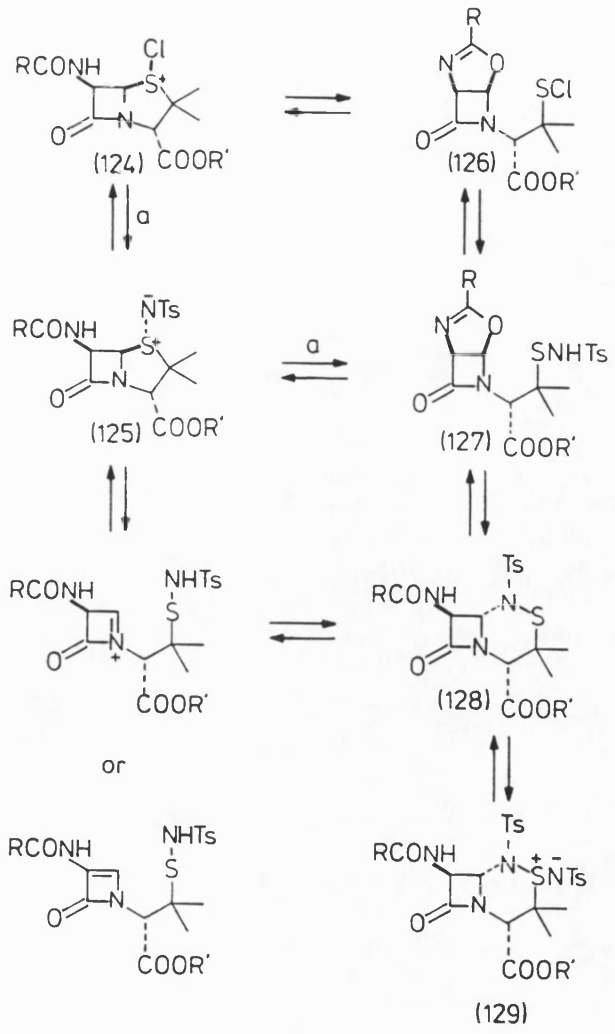


SCHEME 31.

a) sodium N-chlorotoluene-p-sulphonamide trihydrate.

It is thought that chloramine τ reacts at sulphur, forming a S-chlorosulphonium intermediate (124),[75] from which chloride ion may be displaced by toluene-p-sulphonamide ion to form the sulphimide intermediate (125). Ring opening of this species, followed by cyclisation from the α-face would give (128). Conversion of (128) into (129) may involve displacement of chloride from an α-chlorosulphonium intermediate by toluene-p-sulphonamidate ion approaching from the sterically less hindered β-face. Alternative mechanisms involve the oxazolines (126) or (127) as intermediates. It is also possible that the sulphenamide

group in (127) is converted by chloramine T into a S-imide which then cyclises to (129). These possibilities are illustrated in Scheme 32.

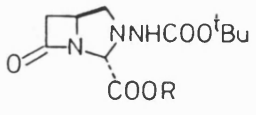


SCHEME 32.

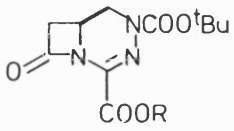
a) sodium N-chlorotoluene-p-sulphonamide trihydrate.

1.1.13 2-aza-3-aza-1-dethiacephams and -cephems.

Stoodley et al. have reported the synthesis of 2-aza-3-aza-1-dethiacephems (133),[76] the unexpected products in an attempted synthesis of 2-azapenem derivatives (130).

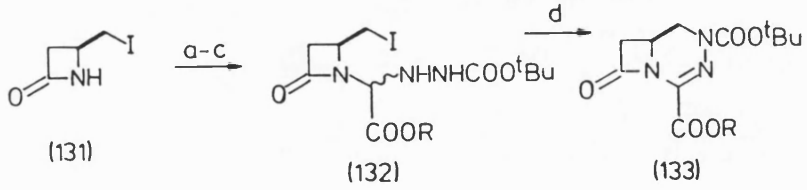


(130)



(133)

Condensation of the known intermediate (131) with a glyoxylic ester, followed by treatment with thionyl chloride/2,6-lutidine then t-butyl carbazate gave the hydrazide (132). Treatment of (132) with silver(I)oxide in acetonitrile gave (133) via an oxidative elimination. (Scheme 33). The free acid (133, R=H) showed no significant antibacterial activity.

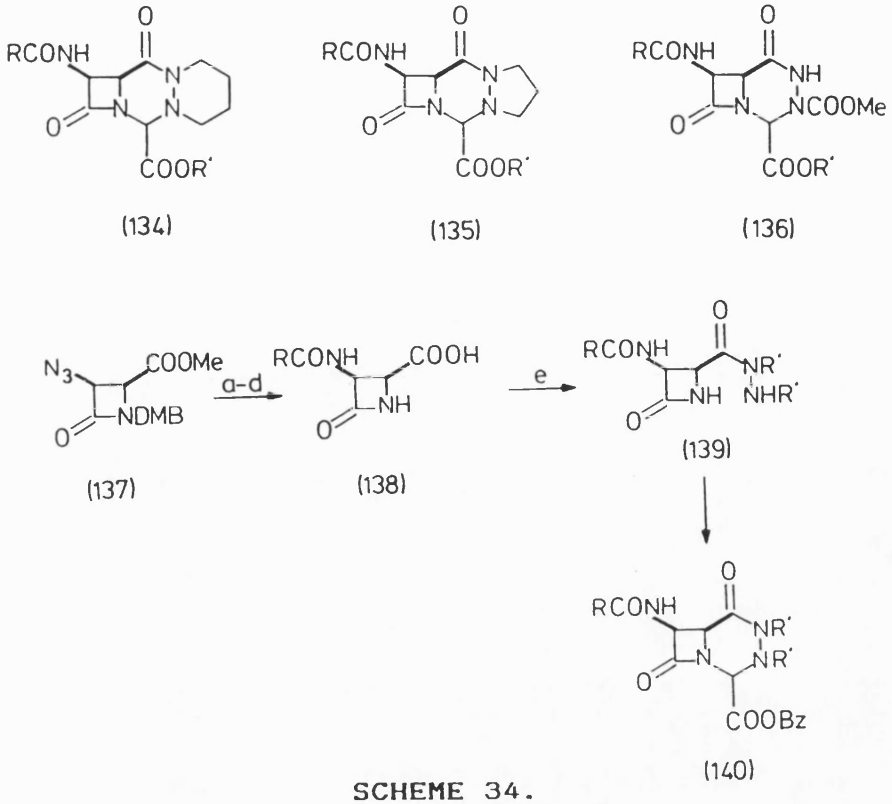


(131) (132) (133)

SCHEME 33.

a) RO_2CCHO: b) $SOCl_2$, 2,6-lutidine: c) ^{t}butyl carbazate: d) Ag_2O, MeCN.

Workers at Smith, Kline & French have reported the synthesis of some bi- and tri-cyclic β-lactams containing a hydrazine moiety (134-136).[20] (Scheme 34).



SCHEME 34.

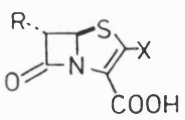
a) $K_2S_2O_8$: b) H_2, Pd/C, TsOH: c) RCOCl, Et_3N, EtOH: d) K_2CO_3, MeOH/H_2O: e) R'NHNHR', DCC, THF: f) BzO_2CCHO, $BF_3.Et_2O$, THF.

The versatile azetidinone (137) was converted into (138) via oxidative cleavage of the dimethoxybenzyl protecting group, reduction of the azide, acylation, then hydrolysis of the ester. Coupling of (138) with a hydrazine gave (139), which was condensed with benzyl glyoxylate giving (140). Hydrogenolysis of the ester (140) in the presence of sodium hydrogen carbonate gave the corresponding sodium salt, some examples of which possessed limited antibacterial activity.

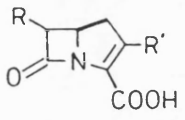
Recent syntheses of hetero-analogues of the cephalosporins have been discussed in this section; in the next section of this introduction the synthesis of hetero-analogues of the penicillins will be discussed.

1.2 Penicillin analogues

The penems (141) and carbapenems (142) need little introduction, for there has been considerable interest in both these structural types in recent years.[10] These compounds in general have proved to be very potent, broad spectrum antibiotics with good stability towards β-lactamases.

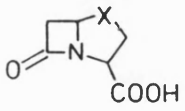


(141)

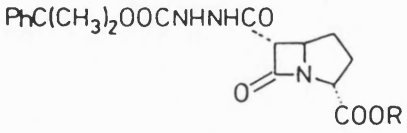


(142)

Preliminary studies on the synthesis of the carbapenams (143, X=CH_2) have also been reported,[77] However it was found that the carbapenam-3-carboxylate (144) was unstable, therefore it is unlikely that the carbapenams will prove to be useful antibacterial agents.

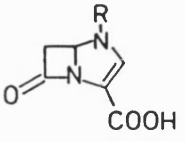


(143)

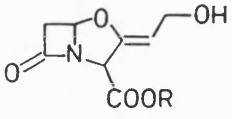


(144)

Analogues have been reported in which the sulphur atom has been replaced by nitrogen: the 1-azapenams (143, X=NR) and azapenems (145).



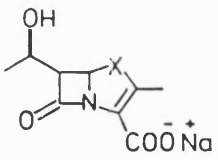
(145)



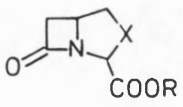
(146)

Analogues in which sulphur has been replaced by oxygen have been synthesised, and the naturally occuring clavulanic acids (146) have recently attracted much interest, as they are potent ß-lactamase inhibitors. An oral formulation of a combination of clavulanic acid and amoxycillin ("Augmentin") is currently marketed by Beecham. A considerable number of analogues have been produced by chemical manipulation of the natural product. The Beecham group have also described the total synthesis of some clavulanic acid derivatives.[7,8]

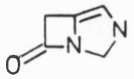
A recent paper reported the synthesis of a selenapenem (147, X=Se), which was found to be active, although less potent than its parent penem (147, X=S).



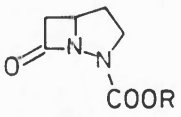
(147)



(148)



(149)

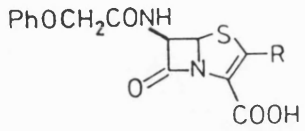


(150)

Analogues in which the sulphur atom is replaced by carbon, and C-2 is replaced by a heteroatom, have also been reported. Examples of isopenams (148, X=S), isoazapenams (148, X=NR) and isooxapenams (148, X=O) have all been synthesised. Syntheses of the 2-azapenem (149) and the 3-azapenam (150) have also been reported.

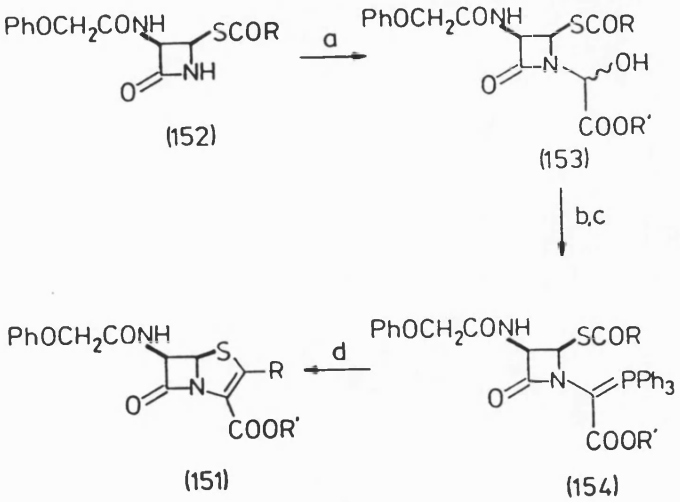
1.2.1 Penems

The penem (151) was synthesised by Woodward in 1975:[79,80] such compounds had not previously been found in nature. The penem (151) proved to have antibacterial activity but was too unstable chemically to be of practical use. However, it was found that the chemically more stable and more easily accessible 6-unsubstituted penems were also active.



(151)

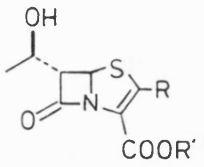
In Woodward's synthesis, (Scheme 35), the chiral azetidinone (152), synthesised from penicillin V, was reacted with a glyoxylic ester to give the hemiaminal (153). This was converted into the phosphorane (154) via the chloride. An intermolecular Wittig condensation between the phosphorane moiety and the carbonyl group of the acylthio substituent gave the penem (151).



SCHEME 35.

a) $R'O_2CCHO$: b) $SOCl_2$, $^iPr_2NEt$: c) PPh_3, dioxane: d) toluene, 100°C.

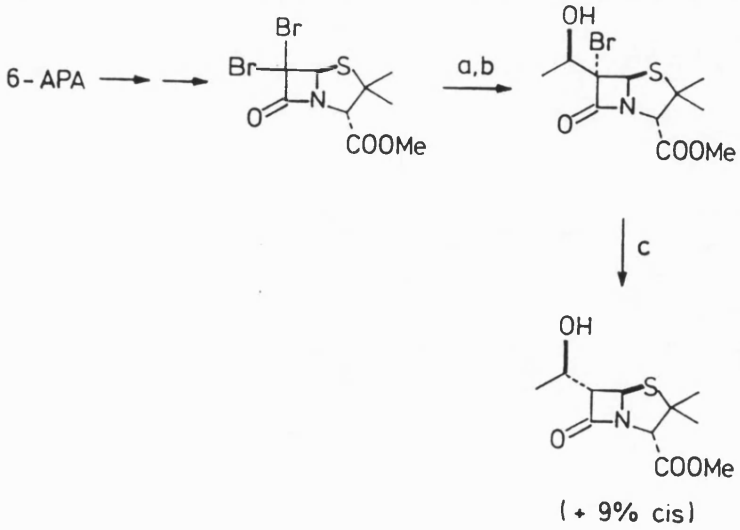
A great research effort was directed towards the synthesis of penems with improved antibacterial activity and β-lactamase stability.[14] In particular it was found that the 6-(R)-hydroxyethyl group in the thienamycin-like 5,6-trans configuration conferred optimum activity. Many pharmaceutical companies have synthesised penem derivatives having varying side chains at C-2 (155), including Merck, Sharp & Dohme,[81] Sankyo,[82] Hoechst,[83] Schering/Plough,[84] and Farmitalia/Carlo Erba.[85]



(155)

R=SEt, $SCH_2CH_2OCONH_2$, CH_2OCONH_2, OC_6H_4F.

6-Aminopenicillanic acid is used in many syntheses as an inexpensive chiral starting material. It is possible to introduce the hydroxyethyl side chain in a stereocontrolled manner, an example of this is the method of Laenza et al.,[81] (Scheme 36).

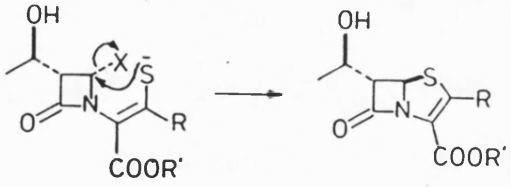


SCHEME 36.

a) MeMgBr: b) CH_3CHO: c) Zn pH 6-7.

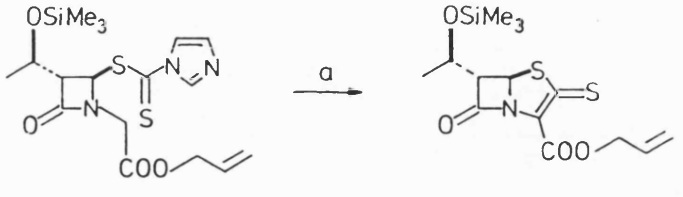
6,6-dibromopenicillanic acid methyl ester reacts with methylmagnesium bromide to give an α-bromoenolate, treatment with excess acetaldehyde gives a mixture of stereoisomeric bromohydrins. The desired isomer can be isolated in 66% yield. Mild reduction with zinc removes the bromine atom and simultaneously results in inversion at C-6, giving the **trans**-hydroxyethyl penam, together with approx. 9% of the **cis** isomer. The hydroxyethylpenam is generally further degraded to a hydroxyethylazetidinone and the thiazoline ring of the penem is then annelated onto this.

There are two strategies of note which have been used for this transformation: firstly, ring closure by nucleophilic attack by sulphur at C-4 of the azetidinone.[82,86,87] (Scheme 37). The **cis**-3(S),4(S) stereochemistry is required to give the **trans**- product, as the ring closure proceeds with inversion.



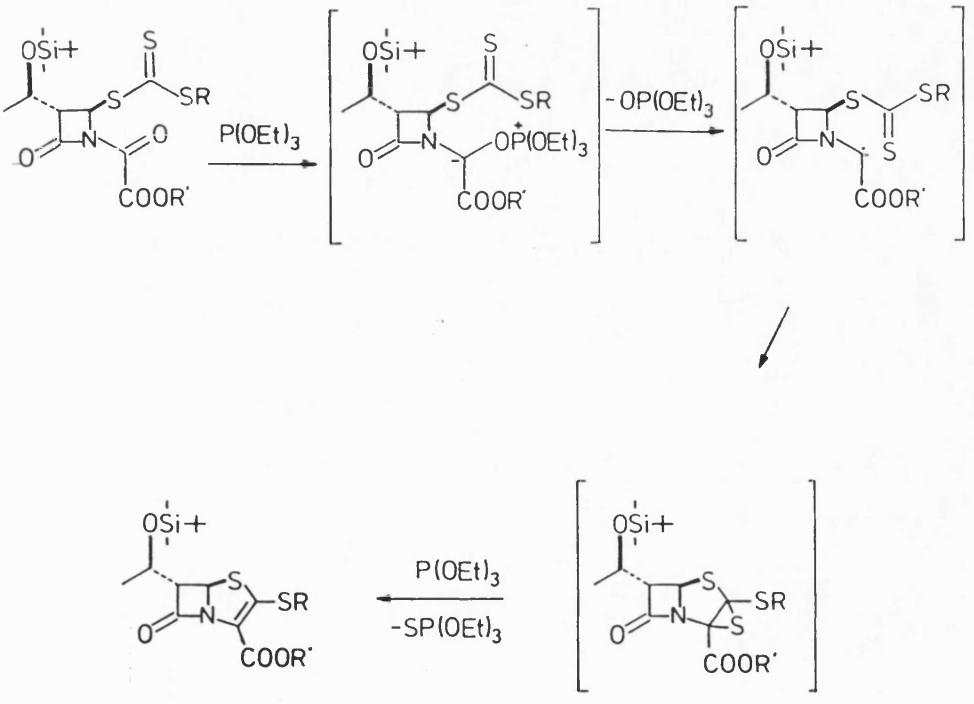
SCHEME 37.

Secondly, cyclisation with formation of the double bond between C-2 and C-3. In Woodward's synthesis, this was accomplished by a Wittig olefination. Recently two new procedures have been introduced: anion cyclisation[88,89] (Scheme 38), and reductive cyclisation of oxalic acid monoamides with trialkylphosphites.[89,90] (Scheme 39)



SCHEME 38.

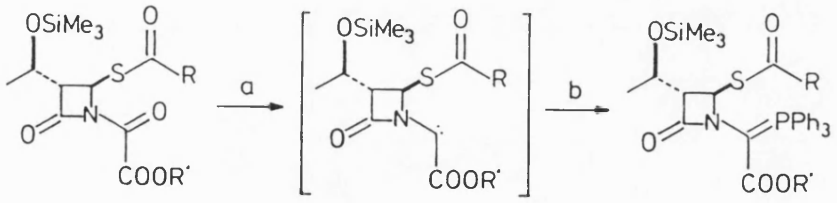
a) 2 equivalents of $LiN(SiMe_3)_2$.



SCHEME 39.

The intermediate carbene adds onto the C=S bond of the trithiocarbonate; the resulting thiiran is desulphurised with a second equivalent of trialkylphosphite. Formation of a phosphorane is possible as a secondary reaction of the carbene with excess phosphite. Hence the desulphurisation is carried out under high dilution and with gradual addition of the phosphite.

If no sufficiently active C=X bond is available, this reaction becomes predominant. This has been exploited as a brief synthetic route to triphenylphosphoranes[91,92] (156), (Scheme 40), giving an alternative to the Woodward procedure.



(156)

SCHEME 40.

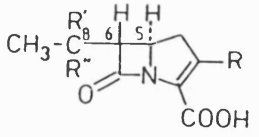
a) $P(OR'')_3$: b) PPh_3.

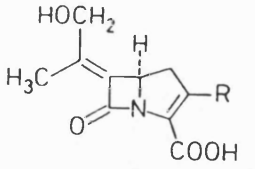
1.2.2 Carbapenems.

Unlike the penems, the carbapenems are found in nature. The thienamycins[93] were the first known examples of such compounds, and to date over thirty carbapenems of natural origin have been discovered. These include epithienamycins, olivanic acids[94], carpetimycins[95], asparenomycins[96], pluracidomycins[97], and carbapenems of the PS group.[98]

Table 1 gives a general outline of the structural differences between the groups. In general the group R is cysteamine or a derivative thereof. In pluracidomycins,

groups such as OSO_3^-, $SOCH_2CO_2^-$, or $SOCH(OH)_2$ are bound to C-2.





asparenomycins

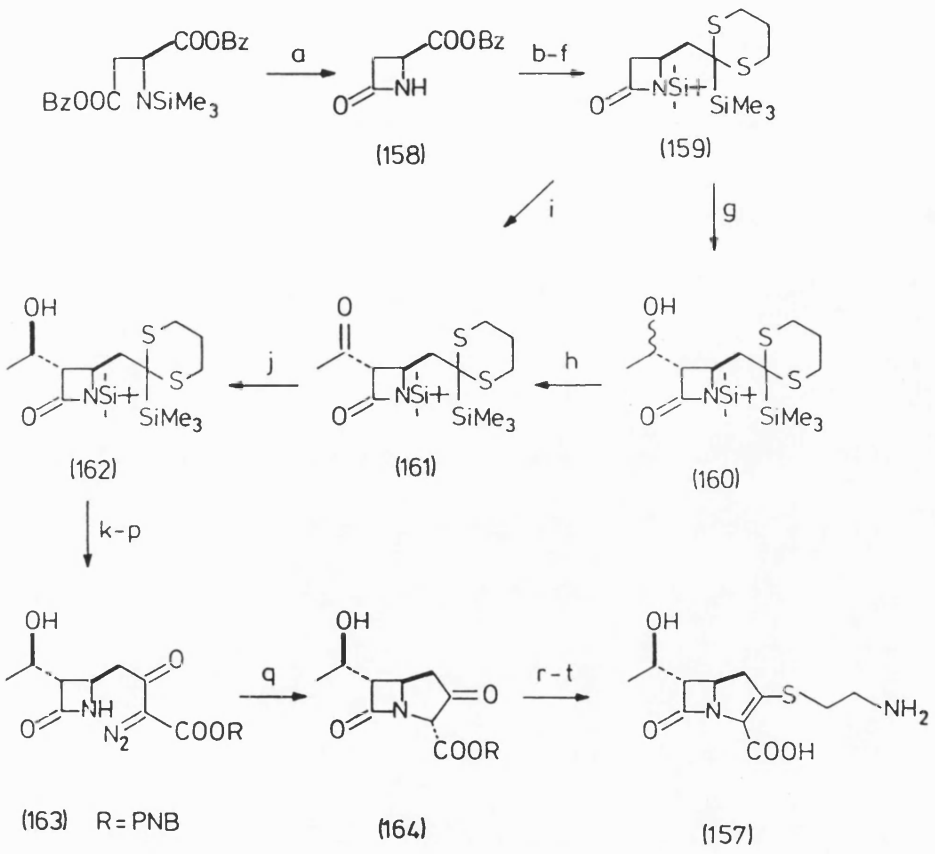
TYPE	R'	R''	H-5/H-6	C-6	C-8
Thienamycins	OH	H	trans	S	R
Olivanic acids	OH	H	cis	R	S
Pluracidomycins	OSO_3^-	H	cis	R	S
Carpetimycins	OH	CH_3	cis	R	
PS group	H	H or CH_3	trans	R	

TABLE 1.

The carbapenems are highly reactive compounds, due to their strained ring system, and are difficult to isolate in large quantities from culture filtrates. Hence synthetic approaches to compounds of this type are currently attracting much interest.[99,100]

Two recent approaches to carbapenems will be outlined here. The first is a total synthesis of (+)-thienamycin[50] (157), which uses L-aspartic acid as a chiron and builds up the carbapenem skeleton in a stereocontrolled way. (Scheme 41) The β-lactam ring closure is achieved at an early stage using t-butyl magnesium chloride. The C-4 side chain of the

resulting azetidinone is homologised via a five-step sequence to give the silyldithiane (159), which is a carboxylic acid analogue.



SCHEME 41.

a) ^{t}BuMgCl, Et_2O, HCl, NH_4Cl: b) $NaBH_4$, MeOH: c) MsCl, Et_3N: d) NaI, acetone: e) ^{t}BDMSCl, Et_3N, DMF: f) 2-trimethylsilyl--1,3-dithian-2-yllithium: g) $LiN(^iPr)_2$, THF, CH_3CHO: h) $LiN(^iPr)_2$, THF, AcIm: i) DMSO/TFAA, Et_3N: j) K-selectride, KI, Et_2O: k) $HgCl_2$, HgO, MeOH/H_2O: l) H_2O_2, MeOH: m) Im_2CO, THF: n) $(PNBO_2CCH_2CO_2^-)_2Mg^{2+}$: o) HCl/MeOH: p) p-$TsN_3$, Et_3N, MeCN: q) $Rh(OAc)_4$, toluene, 80°C: r) $ClPO(OPh)_2$, DMAP, $^iPr_2NEt$, MeCN: s) $HS(CH_2)_2NHCO_2PNB$, $^iPr_2NEt$: t) H_2, Pd/C.

The hydroxyethyl group in (160) is introduced via aldol condensation with acetaldehyde. This reaction is stereoselective with respect to C-3 of the azetidinone; equal proportions of **(8R)**- and **(8S)**- isomers of (160) are produced. The chiral centre at C-8 is removed by Moffat oxidation to (161), which may also be obtained in higher yield from (159) by acetylation.

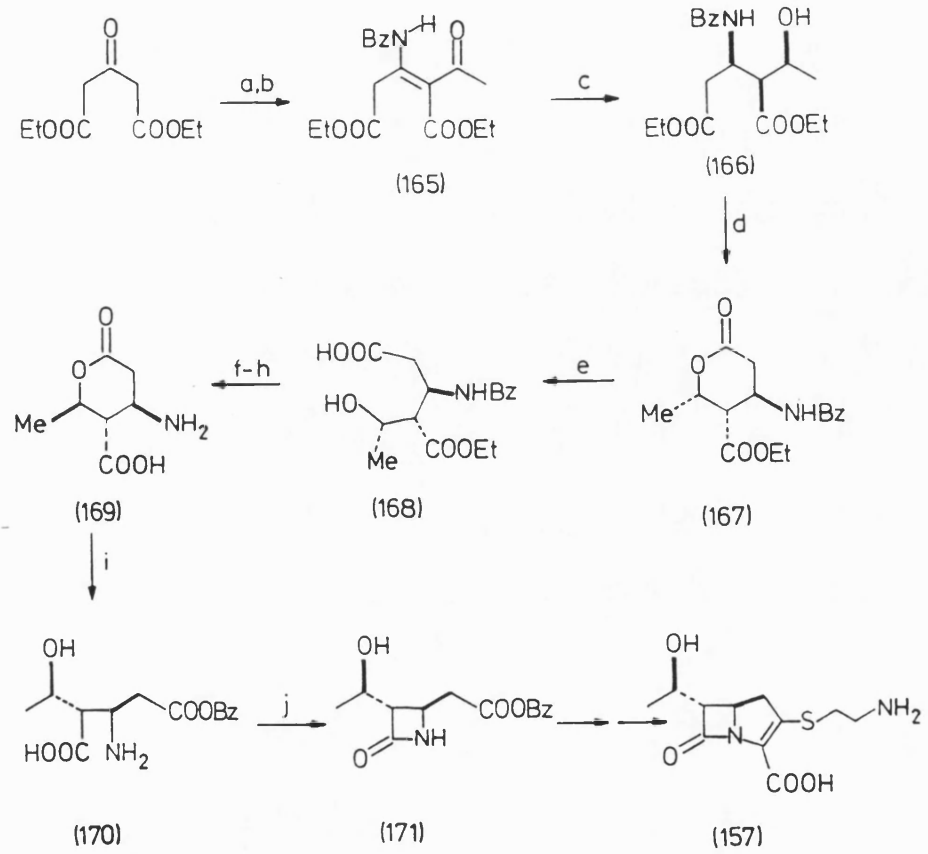
The desired stereochemistry at C-8 is introduced by stereoselective reduction of (161) with K-selectride. A 10% yield of unwanted **(8S)**-diastereomer is produced; this can be recycled. The dithiane (162) is then cleaved and the resulting carboxylic acid is elaborated using a modified Masamune method, and converted by diazo transfer into the carbene precursor (163). Cyclisation is achieved in quantitative yield under mild conditions, in the presence of catalytic amounts of rhodium (II) acetate, to give the 2-oxo-carbapenam (164). The cysteaminyl side chain is introduced via activation with diphenylphosphonyl chloride, giving (+)-thienamycin (157). Note that during the entire synthesis the hydroxy group at C-8 does not need to be protected.

One drawback of this synthesis is the time-consuming and expensive homologisation of (158). This has been eliminated in a later modification[101] of this route, which also provides stereocontrol at C-8. (Scheme 42)

Starting from diethyl acetonedicarboxylate, the enamine (165) is prepared. In (165) a strong hydrogen bond forces the acetyl group into the **cis** position relative to the benzylamino group. The racemic diester (166) is prepared by stereoselective reduction with sodium cyanoborohydride. The diester (166) contains all but two of the atoms required for the carbapenem skeleton, and all the substituents except the

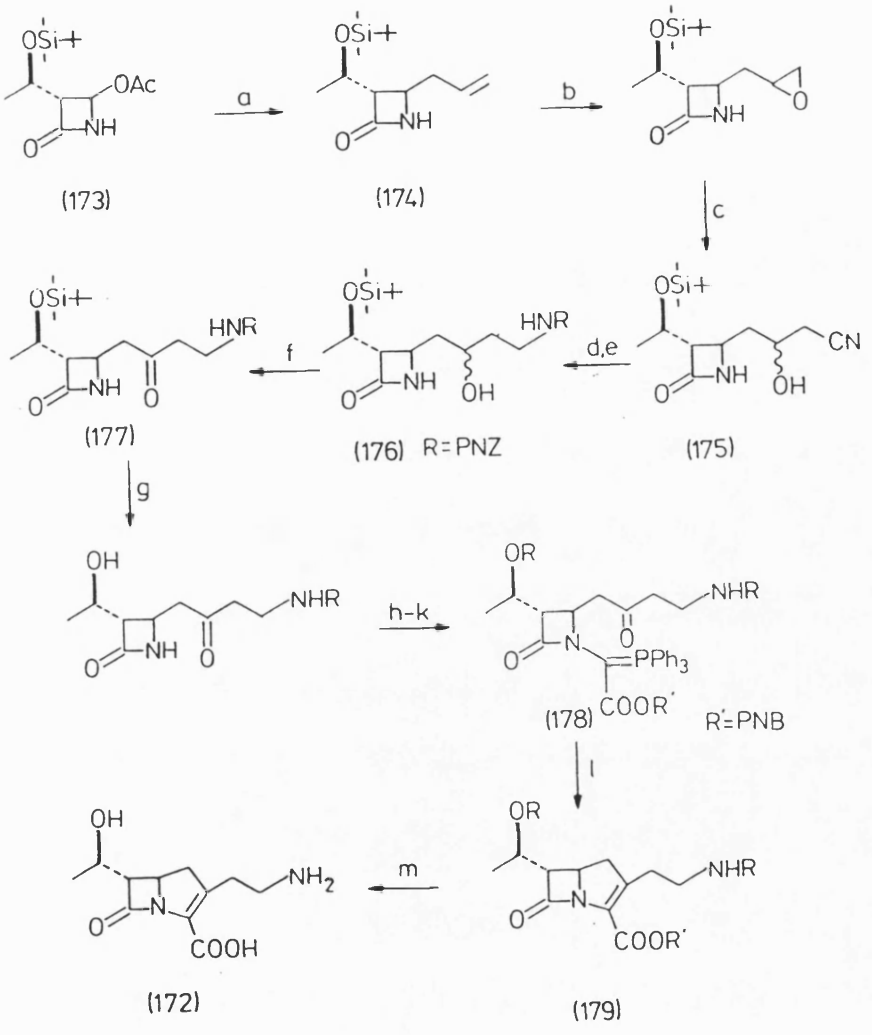
OH group have the desired relative configuration.

The sequence (167)-(169) achieves the epimerisation of the hydroxyethyl group via an intramolecular Mitsunobu reaction. The lactone (169) is opened to give the benzyl ester (170) by solvolysis in benzyl alcohol, and is converted into the azetidinone (171) with DCCI. The total synthesis of thienamycin is then completed as in the previous scheme.



SCHEME 42.

a) $BzNH_2$, mol sieves: b) ketene: c) $NaBH_3CN$, AcOH: d) HCl, CH_2Cl_2: e) $NaHCO_3/H_2O$: f) PPh_3, DEAD, THF: g) conc. HCl, reflux: h) H_2, $Pd(OH)_2/C$: i) $PhCH_2OH$, 70°C: j) Et_3N, DCCI, $PhCH_2OH$, 55°C.



SCHEME 43.

a) tetraallylstannane, CH_2Cl_2: b) mCPBA: c) KCN, DMF, H_2O: d) H_2, PtO_2: e) PNZCl, $^iPr_2NEt$: f) PCC, CH_2Cl_2: g) $BF_3.Et_2O$, MeCN: h) PNZCl, DMAP: i) $PNBO_2CCHO$: j) $SOCl_2$, py: k) Ph_3P, py: l) benzene, reflux: m) H_2, Pd/C, phosphate buffer.

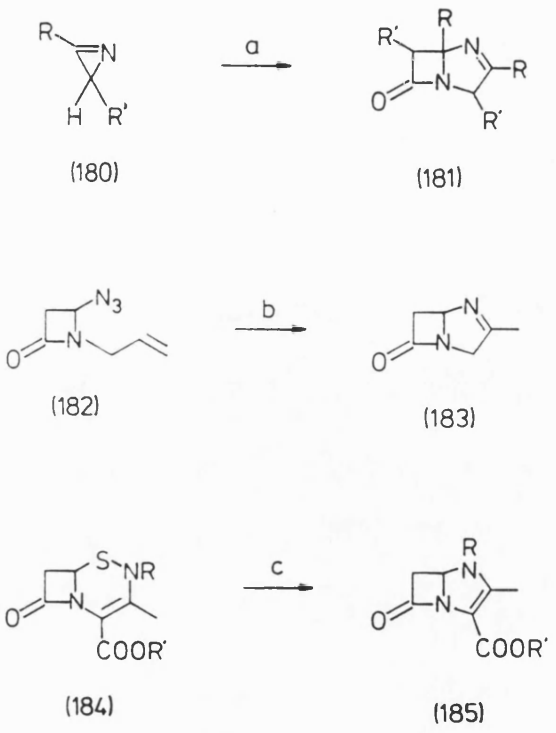
A recent Sankyo synthesis of dethiathienamycin[102] (172) is also interesting, as it employs an analogous intramolecular Wittig procedure, similar to that used in penem synthesis. In contrast to the above methodology, this is a semi-synthetic route. (Scheme 43)

The azetidinone starting material (173) was synthesised in 7 steps from 6-aminopenicillanic acid[103]. Reaction of azetidinone (173) with tetraallylstannane gave the 4-allylazetidinone (174) in 86% yield. Epoxidation and regiospecific opening of the epoxide with cyanide gave the cyanoalcohol (175). The cyano moiety was reduced by catalytic hydrogenation, and the resulting amino function was protected as the p-nitrobenzylcarbamate, yielding (176) as a mixture of diastereomers. Oxidation with pyridinium chlorochromate gave the keto compound (177). Removal of the silyl protecting group and re-protection with the p-nitrobenzyloxycarbonyl group was followed by treatment with p-nitrobenzyl glyoxylate, chlorination with thionyl chloride then formation of the phosphorane (178), as in the standard Woodward procedure[79]. Cyclisation in benzene under reflux yielded the protected carbapenem (179), which was deprotected via catalytic hydrogenation to give dethiathienamycin (172).

1.2.3 1-azapenams and 1-azapenems.

Following Woodward's synthesis of penems, various groups have tried to synthesise 1-aza analogues. Alper *et al.*[104] obtained (181) by treatment of (180) with palladium catalyst in the presence of carbon monoxide. Nagakura[105] obtained the azapenam (183), which lacks a carboxylic acid function at C-3, by heating the azetidinone (182) under reflux in toluene. Workers at Hoechst synthesised the 2-aza-1-thiacephem (184), which lacks an acylamido side chain at C-7, using similar methodology to that described later in this thesis. Treatment

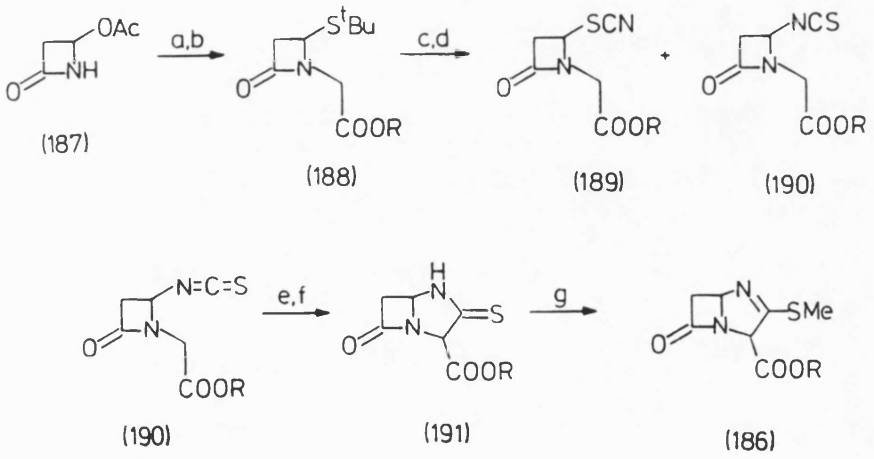
of (184) with triphenylphosphine led to the azapenem (185), however desulphurisation was accompanied by racemisation. Attempted deprotection of (185) by catalytic hydrogenation was unsuccessful.



a) $Pd(PPh_3)_4$, CO: b) toluene, reflux: c) PPh_3.

A more recent Hoechst paper[106] described the synthesis of a racemic 2-thiomethylΔ^1-azapenem (186) from 2-acetoxy-azetidinone (187). (Scheme 44). Displacement of the acetoxy group with t-butyl thiolate, then N-alkylation with α-bromomethyl acetate gave the substituted azetidinone (188). Chlorinolysis with chlorine in carbon tetrachloride gave the 4-chloro-azetidinone which, without purification, was treated with potassium thiocyanate at 20°C, yielding a mixture of the thiocyanate (189) and isothiocyanate (190). It was found that heating this mixture at 40°C caused isomerisation of one compound into the other. This compound was assumed to be the isothiocyanate, on the basis of the known thermal rearrangement of thiocyanates to

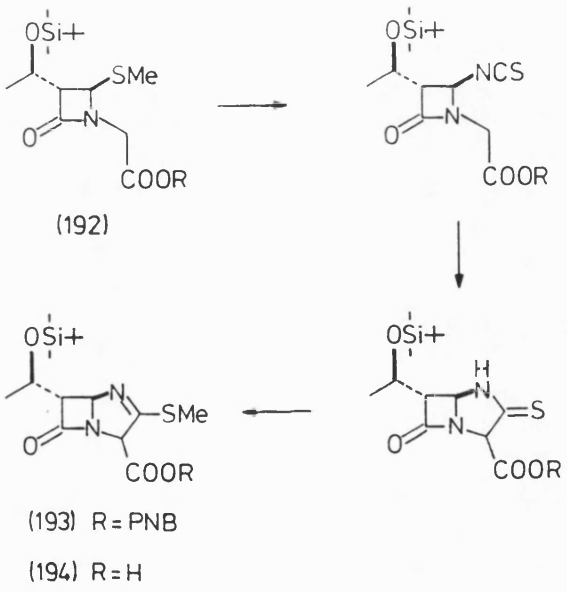
isothiocyanates[107], and the subsequent reaction of the compound. Generation of the lithium enolate under very mild conditions with lithium hexamethyldisilazide, then quenching with acetic acid, gives the bicyclic 2-thioxo-1-azapenam (191). S-alkylation of (191) is achieved using methyl iodide, yielding the 2-methylthio-Δ^1-azapenem (186). An nmr study in various solvents showed no evidence of the presence of Δ^2-isomer. The deprotection of the p-nitrobenzyl ester of the azapenam (186, R=PNB) was carried out by catalytic hydrogenation then treatment with potassium hydrogen carbonate; giving the salt (186, R=K), which showed no interesting biological properties.



SCHEME 44.

a) sodium^{t}butylthiolate, EtOH: b) $BrCH_2CO_2Me$ or $BrCH_2CO_2PNB$, DMF, K_2CO_3: c) Cl_2, CCl_4: d) KSCN, MeCN, 40°C: e) $LiN(SiMe_3)_2$, THF, -78°C: f) AcOH: g) MeI, MeCN, K_2CO_3.

A recent paper by the Sankyo group reports a synthesis[108] of the chiral 2-methylthio-Δ^1-azapenem (194) (Scheme 45), based on the Hoechst methodology. The threonine-derived azetidinone (192) is used as starting material. Again, none of the Δ^2-azapenem was observed, and an X-ray crystal structure confirmed the absolute stereochemistry of (193); it is assumed that cyclisation gives the thermodynamically more stable diastereomer. The free acid (194) showed only weak antibacterial activity.



(192)

(193) R = PNB

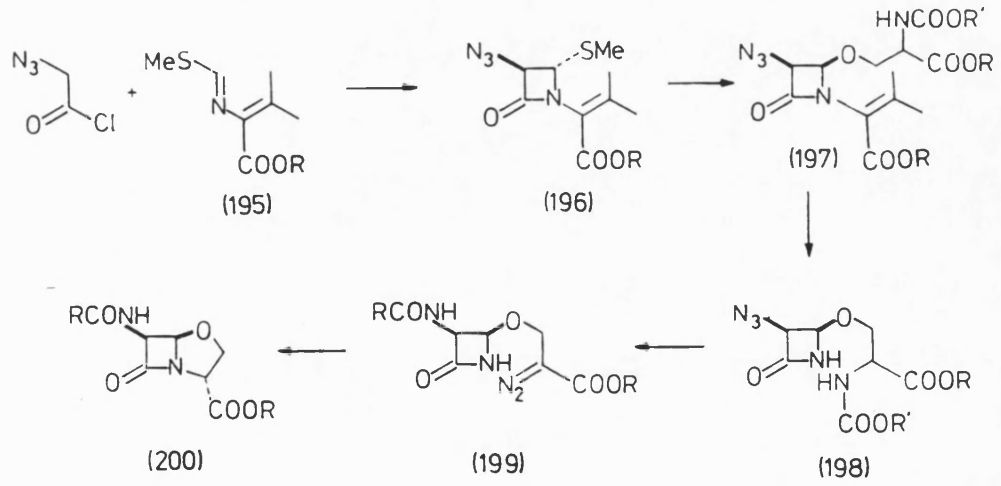
(194) R = H

SCHEME 45.

1.2.4 1-oxapenams.

The oxa analogue of penicillin V has been prepared in optically active form starting from penicillin V methyl ester.[109] It was found to be considerably less active than penicillin V against a range of bacteria. The Merck group have reported a similar synthesis of 1-oxapenams, involving C-3 to N-4 ring closure.[110] (Scheme 46.) The azetidinone

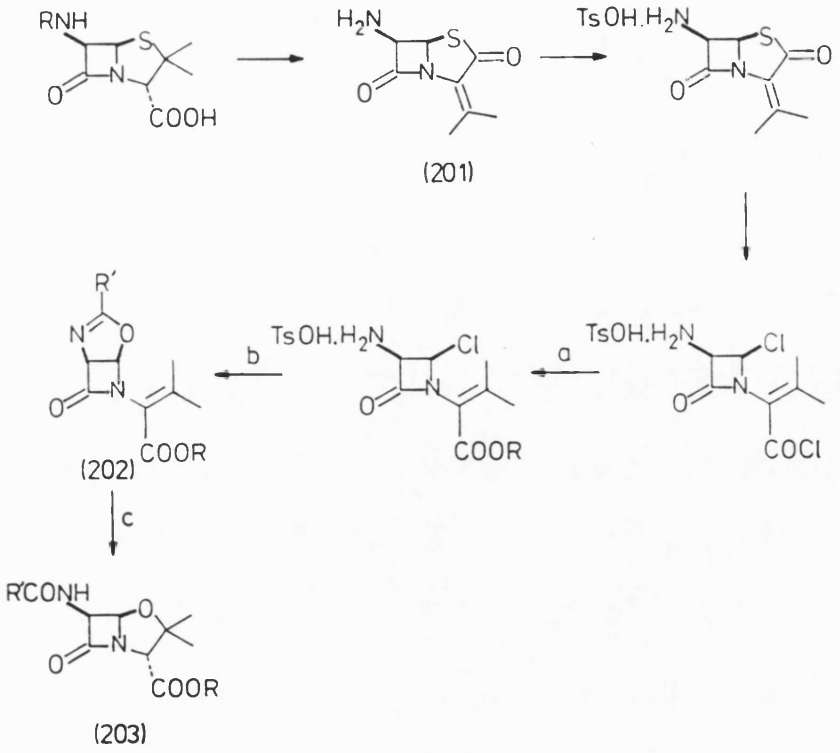
precursor (196) is produced by reaction of azidoacetyl chloride with the imine (195). Chlorination of (196) gave a mixture of **cis**- and **trans**-4-chloro derivatives which were displaced with N-t-butoxycarbonylserine benzyl ester to give (197). The substituent on the β-lactam nitrogen was removed by potassium permanganate oxidation, and the desired **cis**-isomer (198) was separated from the resulting mixture. Reduction of the azide, then acylation, followed by removal of the t-butyl group and diazotisation of the amine gave (199). Cyclisation to (200) was achieved by intramolecular carbene insertion using rhodium acetate as catalyst.



SCHEME 46.

An alternative approach, utilising the oxazoline (202) was achieved by Wolfe[111,112]. (Scheme 47). In this reaction sequence the ring opening method used is that of Kukolja[113]. Starting with a natural penicillin, the 6-aminoanhydropenicillanic acid (201) is formed, and the

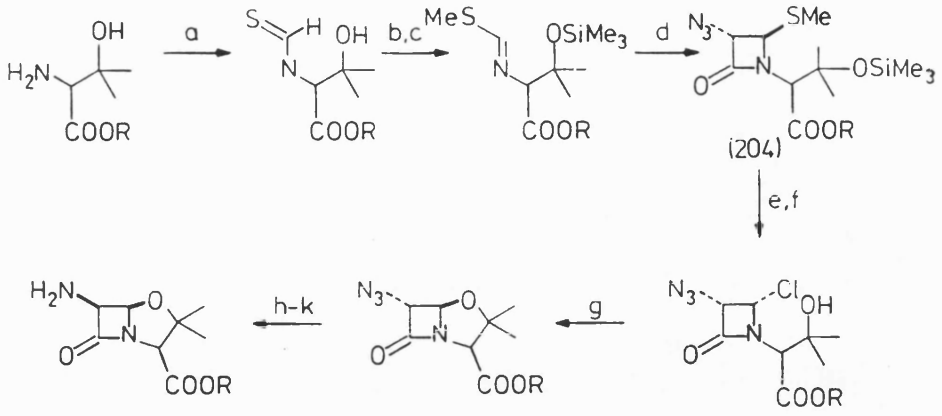
p-toluenesulphonic salt is transformed to the 1-oxapenam (203).



SCHEME 47.

a) ROH: b) R'COCl, Al_2O_3: c) R''SLi, HMPA.

A total synthesis of racemic 1-oxapenams utilising a [2+2] cycloaddition reaction in the formation of the azetidinone (204), has been patented[114,115]. (Scheme 48).



SCHEME 48.

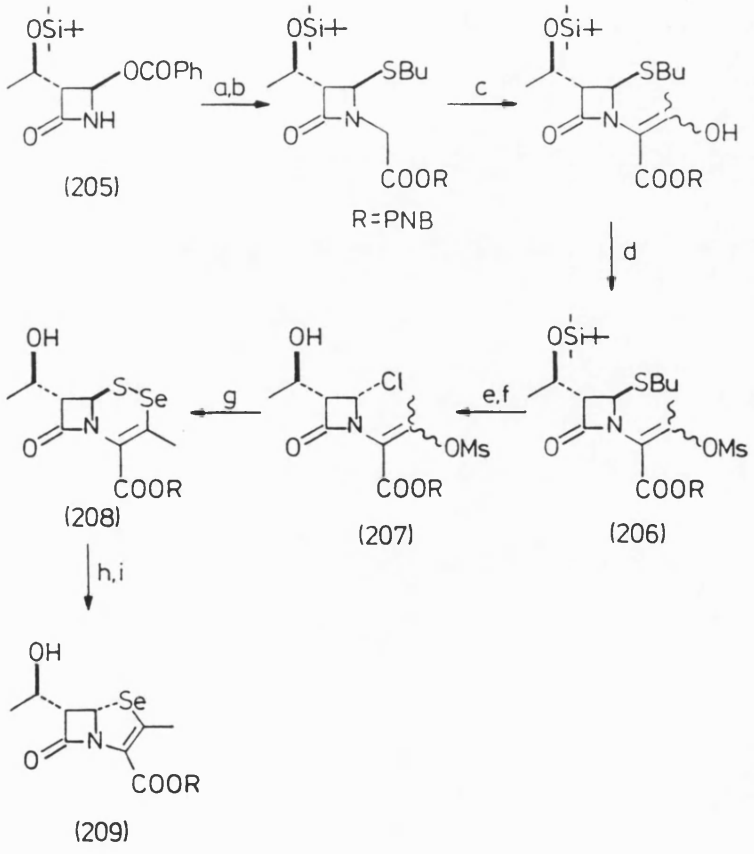
a) HCSOEt, H_2S: b) TMSCl, TMS_2NH: c) MeI, K_2CO_3, acetone: d) N_3CH_2COCl, Et_3N, CH_2Cl_2: e) Cl_2, CCl_4: f) HCl, H_2O, dioxane: g) Ag_2OSCF_3, THF: h) H_2, Pt_2O: i) PhCHO, $MgSO_4$: j) PhLi, THF, DMF: k) AcOH, 2,4-DNP, TsOH.

1.2.5 1-dethia-1-selenapenems.

A recent paper by Perrone et al. reported the synthesis of the first bicyclic selena-β-lactams.[116] (Scheme 49). These were synthesised via desulphurisation of 2-selenacephems: analogous to the Hoechst desulphurisation of 2-azacephems to give 1-azapenems.

Conversion of the threonine-derived benzoyloxyazetidinone[117] (205) into the thioether (206) was achieved by sequential displacement with butylmercaptan, N-alkylation with p-nitrobenzyl iodoacetate, C-alkylation with $LiN(SiMe_3)_2$ and acetyl chloride, then mesylation of the enol. Chlorinolysis of 4-alkyl thioazetidinones can be reagent approach controlled when the hydroxyethyl group at C-3 is unprotected. Hence the deprotection of (206) prior to chlorinolysis, affords the 4α-chloroazetidinone (207).

Treatment of (207) with sodium selenide yielded the 2-selenacephem (208), which was desulphurised with triphenylphosphine with inversion of stereochemistry at C-5, giving (209, R=PNB). Both cyclisation and desulphurisation were reported to proceed in low yield. Deprotection of the ester group at C-3 was carried out by reduction with iron powder in a buffered medium, and the sodium salt (209, R=Na) was isolated after ion exchange and reverse phase chromatography.

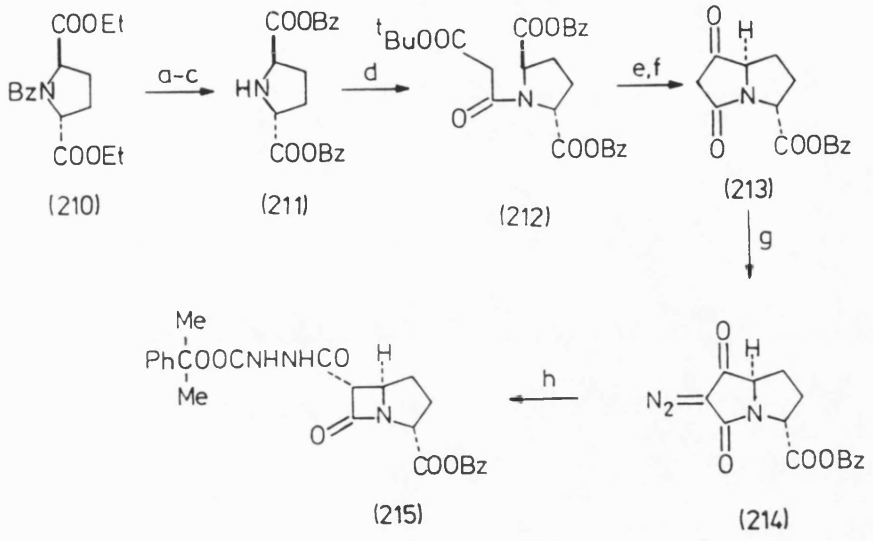


SCHEME 49.

a) BuSH, NaH, DMF: b) PNB iodoacetate, Cs_2CO_3, MeCN: c) $LiN(SiMe_3)_2$, CH_3COCl, THF: d) MsCl, Et_3N, CH_2Cl_2: e) $BF_3.Et_2O$, MeCN: f) Cl_2, CCl_4: g) Na_2Se: h) PPh_3: i) Fe, NH_4Cl, THF/H_2O.

1.2.6 Carbapenams.

Very few synthetic routes towards the carbapenam skeleton have been published. One example is that of Lowe, who synthesised the carbapenam (215) via a photolytic Wolff rearrangement.[77] (Scheme 50).



SCHEME 50.

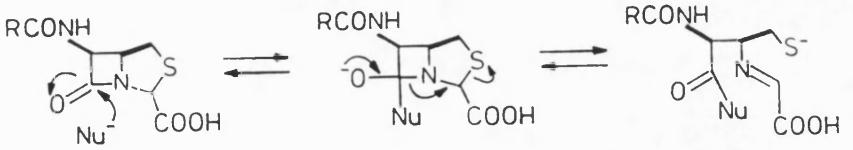
a) H_2, Pd/C: b) H_2O, reflux: c) TsOH, BzOH, benzene: d) $^tBuO_2CCH_2CO_2H$, DCCI: e) NaH, benzene, reflux: f) toluene, reflux: g) $MeSO_2N_3$, Et_3N: h) h , $PhCMe_2O_2CNHNH_2$.

cis-N-Benzyl-2,5-bisethoxycarbonyl pyrrolidine was isomerised to the **trans**-stereoisomer (210) by treatment with base; hydrogenolysis then ester hydrolysis and re-esterification gave the **trans**-dibenzyl ester (211). This was then coupled to t-butyl hydrogen malonate using DCCI, to give (212). Cyclisation was achieved with sodium hydride in benzene under reflux and the t-butoxycarbonyl group was removed by heating in toluene under reflux. The resulting pyrrolizidinedione (213) underwent rapid diazo-exchange when treated with methanesulphonyl azide in the presence of

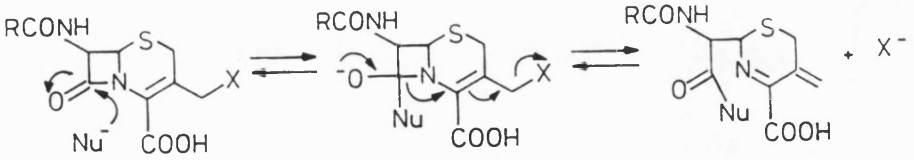
triethylamine, yielding (214). Irradiation of (214) in the presence of 1 equivalent of β-methylphenethyl carbazate gave the unstable **trans**-1-carbapenam (215).

1.2.7 2-thia-1-dethiapenams.

As part of the search for new, active β-lactam analogues, workers at Smith, Kline & French became interested in the 1-dethia-2-thiapenams (216). It was hoped that the sulphur atom in the 2-position would enhance the reactivity of the β-lactam amide linkage by providing a facile mechanism for cleavage, analogous to the role of the 3'-substituent in cephalosporins (217). (Scheme 51)



(216)

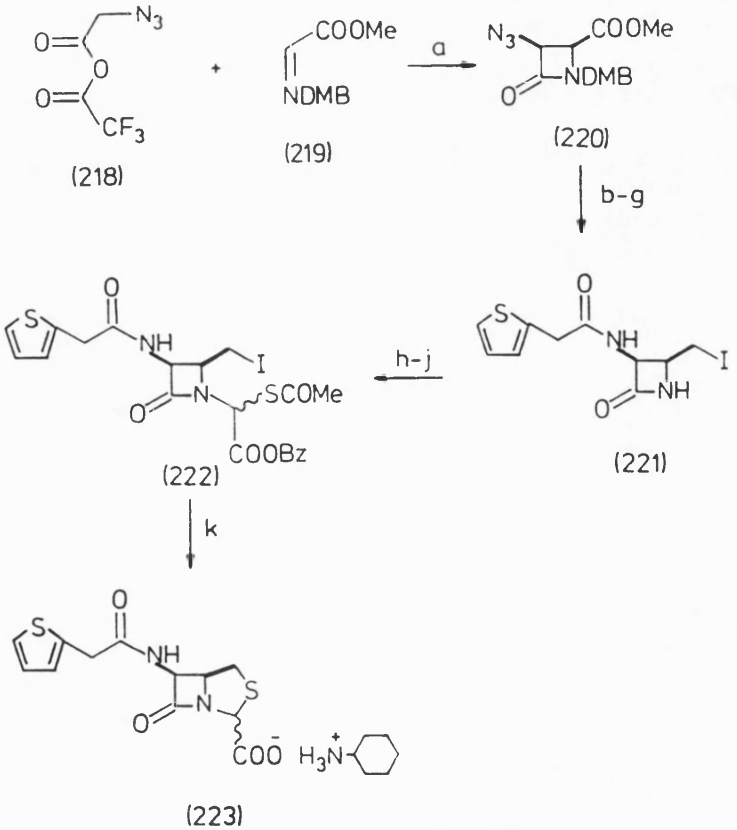


(217)

SCHEME 51.

The total synthesis of 1-dethia-2-thiapenams due to the SK+F group[118] is outlined in Scheme 52. The chiral azetidinone (220), was prepared from the imine (219) and the mixed anhydride of azidoacetic acid and trifluoroacetic anhydride

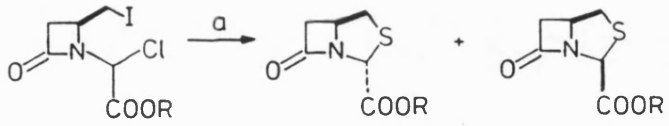
(218). This monocyclic β-lactam has proved to be a versatile intermediate in the synthesis of bicyclic β-lactam analogues. Elaboration of the C-3 side chain and N-deprotection, followed by reduction of the C-4 ester group, tosylation of the resulting alcohol, and displacement of tosylate by iodide, resulted in the 4-iodomethyl derivative (221). This was converted into the thioacetate (222) via the standard glyoxylate procedure, then chlorination and subsequent displacement with potassium thioacetate. Treatment with excess cyclohexylamine liberated the thiol which cyclised spontaneously, and also cleaved the benzyl ester, giving the 1-dethia-2-thiapenam (223), which was found to be active against gram-negative bacteria.



SCHEME 52.

a) Et_3N: b) H_2, Pd/C, EtOH: c) thienylacetyl chloride, Et_3N: d) $K_2S_2O_8$, Na_2HPO_4, MeCN: e) $NaBH_4$, H_2O/THF: f) TsCl, py: g) NaI, acetone: h) BzO_2CCHO, $BF_3.Et_2O$: i) $SOCl_2$, py, THF: j) KSCOMe, DMF/THF: k) cyclohexylamine, CH_2Cl_2.

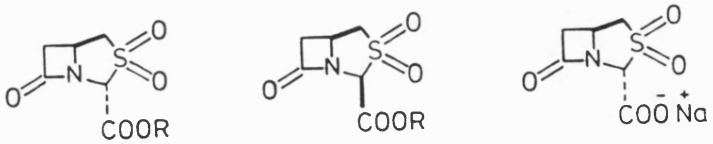
In a more recent paper Stoodley *et al.* reported a modification of the above route,[119] (Scheme 53) involving treatment of the azetidinone (224) with hydrogen sulphide and triethylamine to give a mixture of the diastereomeric 1-dethia-2-thiapenams (225) and (226), which were separable by chromatography. Stoodley also reports the oxidation of (225) and (226) with potassium permanganate in aqueous acetic acid to give the corresponding dioxides (227) and (228). (227) was subjected to hydrogenolysis in the presence of sodium hydrogen carbonate, and the resulting sodium salt (229) was tested as a β-lactamase inhibitor. The 1-thia analogue of this compound, although devoid of useful antibacterial activity, is a β-lactamase inhibitor, and it was hoped that (229) would also exhibit inhibitory properties. However, no such effect was observed.



(224) (225) (226)

SCHEME 53.

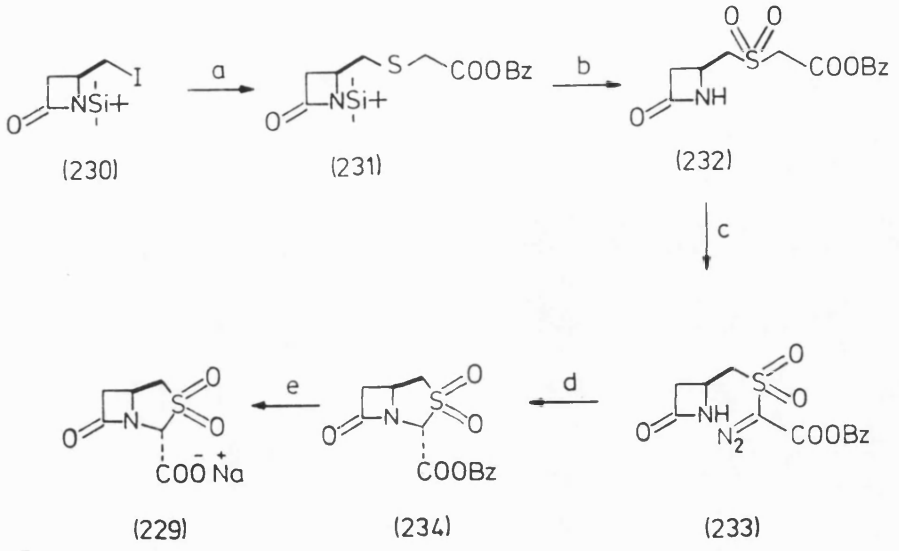
a) H_2S, Et_3N.



(227) (228) (229)

Brennan has described an alternative route to (229),[120] which is analogous to a route used in carbapenem synthesis and

proceeds via C-3 to N-4 bond closure.[101a] (Scheme 54) The N-protected 4-iodomethylazetidinone (230) was treated with benzyl mercaptoacetate to yield the sulphide (231); this was simultaneously oxidised and deprotected using "oxone" [121] to yield sulphone (232). Diazo transfer gave diazoester (233) which cyclised on decomposition to give the protected bicyclic dioxide (234).



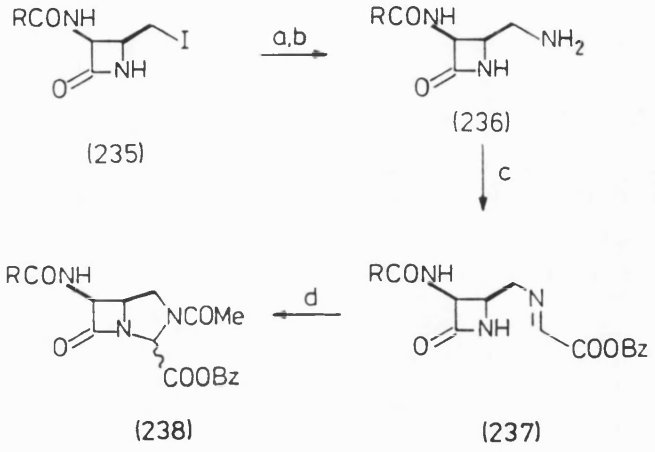
SCHEME 54.

a) benzyl mercaptoacetate, NaH, THF: b) "oxone", MeOH: c) p-carboxybenzenesulphonyl azide, Et_3N, MeCN: d) $Rh(OAc)_2$, benzene, 80°C: e) H_2, Pd/C, $EtOAc/H_2O$, $NaHCO_3$.

1.2.8 2-aza-1-dethiapenams and 2-aza-1-dethiapenems.

A SK+F synthesis of 2-aza-1-dethiapenams[122] involves the elaboration of a chiral azetidinone precursor, then cyclisation involving C-3 to N-4 bond formation. (Scheme 55)

Synthesis of an azetidinone analogous to the precursor (235) has already been described in a previous section.[52] Reaction of (235) with sodium azide followed by catalytic hydrogenation led to the primary amine (236). Condensation with benzyl glyoxylate gave the imine (237) which cyclised to the 2-aza-1-dethiapenam (238) on treatment with acetyl chloride. This methodology provided some precedent for the azetidinone-imine condensation reactions described later in this thesis.

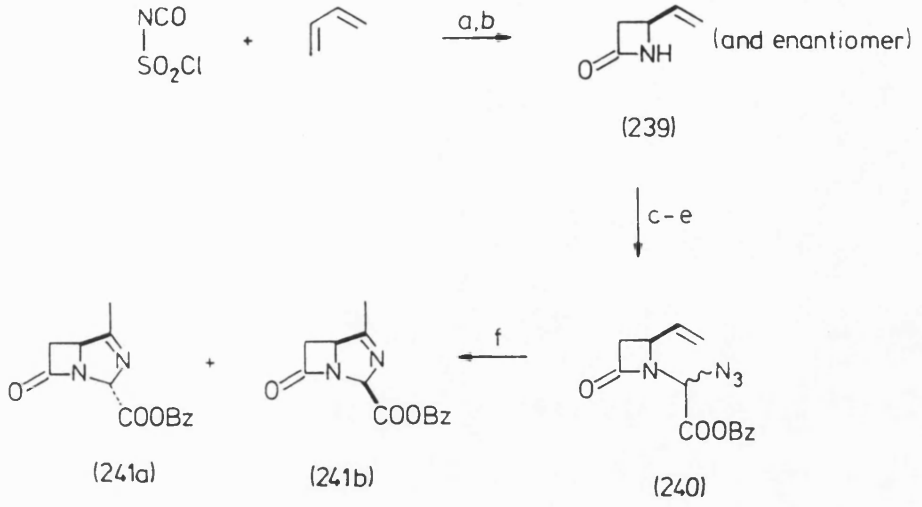


SCHEME 55.

a) NaN_3, DMF: b) H_2, 10% Pd/C: c) BzO_2CCHO, $MgSO_4$, CH_2Cl_2: d) CH_3COCl, py.

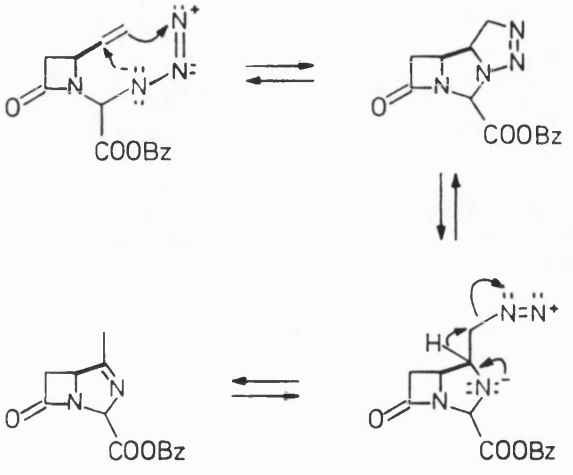
The synthesis of various 2-aza-1-dethiapenems has been reported by both Nagakura[105] and the Beecham group[123] (Scheme 56). The same synthetic methodology is employed by both groups: cyclisation involves 1,3-dipolar cycloaddition of an azide to a vinyl group, with subsequent loss of nitrogen. The azetidinone precursor (239) is produced as a mixture of enantiomers via [2+2] cycloaddition of butadiene to chlorosulphonyl isocyanate.[124] Successive treatment of the β-vinyl enantiomer with benzyl glyoxylate, mesyl chloride and sodium azide gave the azide (240) as a diastereomeric

mixture. When the azide (240) was heated under reflux in toluene, the R-epimer readily cyclised to the α-carboxy product (241a), whereas the S-epimer was only partially cyclised to (241b). The mechanism of the cyclisation reaction is thought to proceed as in Scheme 57.[125]



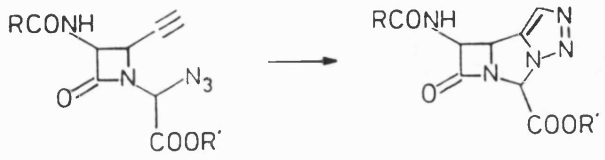
SCHEME 56

a) Et_2O, reflux: b) H_2O: c) BzO_2CCHO, benzene: d) MsCl, Et_3N: e) NaN_3: f) toluene, reflux.



SCHEME 57.

The closely-related triazolopenams (243) have been prepared by Beecham [126] from the corresponding 4-alkynyl azetidinones (242) (Scheme 58).

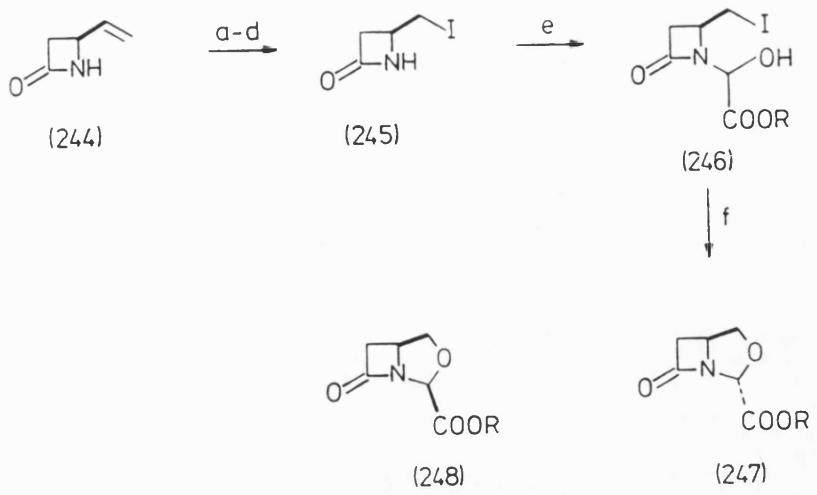


(242) (243)

SCHEME 58.

1.2.9 2-oxa-1-dethiapenams.

Compounds of this type were first synthesised by Stoodley[127] via displacement of iodide ion at C-1 by oxygen. (Scheme 59) The synthesis of the vinylazetidinone (244) is described elsewhere in this report.[128] Conversion of (244) into the 4-iodomethyl azetidinone (245) was achieved via a four-step sequence. Hydroxyalkylation of (245) with a glyoxylate ester produced (246) as a 1:1 mixture of diastereomers. Treatment with sodium hydride produced the 2-oxa-1-dethiapenam (247). It is assumed that two diastereomeric products (247) and (248) are formed initially, but under the reaction conditions (248) is isomerised to the thermodynamically more stable diastereomer (247).

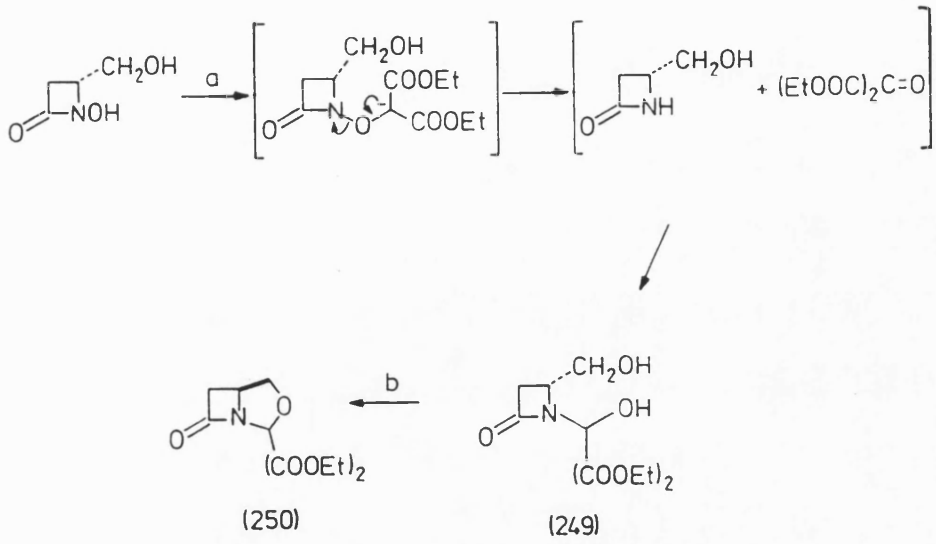


SCHEME 59

a) O_3, MeOH: b) $NaBH_4$: c) TsCl, py: d) NaI, acetone: e) RO_2CCHO: f) NaH.

The Bayer group have reported a modification of this route,[129] involving fewer synthetic steps, and have deprotected the ester (247). The corresponding sodium salt was found to be a β-lactamase inhibitor, but is almost devoid of antibacterial activity. The 4-iodomethyl azetidinone (245) was prepared directly via [2+2] cycloaddition of allyl iodide and chlorosulphonyl isocyanate, according to the method of Tanaka et al. [130] Reaction with allyl glyoxylate then intramolecular O-alkyl- ation in THF in the presence of 2 equiv. HMPA and 1 equiv. n-butyl-lithium gave a mixture of diastereomeric allyl esters (247, R=allyl) and (248, R=allyl), which could be separated by chromatography. The allyl ester (247, R=allyl) was converted to the corresponding sodium salt using palladium(0) catalysis.[131]

Miller et al. have described an alternative method to 3,3-disubstituted 2-oxa-1-dethiapenams,[60] involving an anionic NOC⁻ to NCO⁻ rearrangement as a key step in the synthesis of the carbinolamine (249), and cyclisation of (249) under Mitsunobu conditions to give the 2-oxa-1-dethiapenam (250). (Scheme 60).



(250) (249)

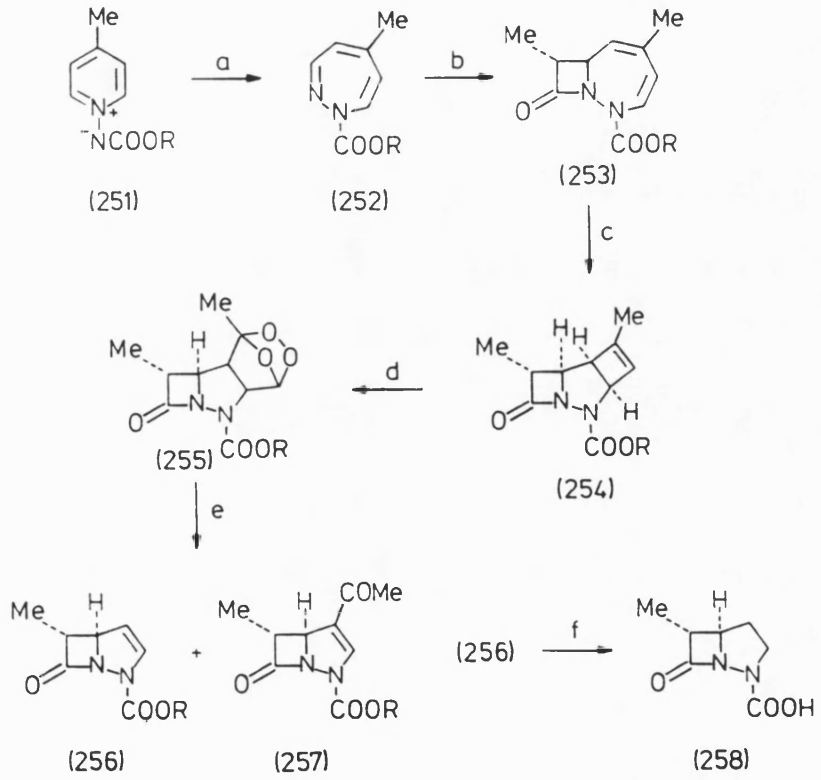
SCHEME 60.

a) $BrCH(CO_2Et)_2$, KOH, DMSO: b) DEAD, PPh_3.

1.2.10 3-aza-1-dethiapenams.

Tschamber et al. have recently reported the synthesis of 3-aza-1-dethiapenams[132] (258) by a photochemical route. (Scheme 61). Starting from the N-iminopyridinium ylide (251), which is easily obtained from 4-picoline, ultra-violet irradiation gave the corresponding 1,2-diazepine (252), to which methylketene was added stereospecifically according to a known procedure,[133] giving the azetidinodiazepine (253) in 94% yield. Ultra-violet

irradiation of (253) gave the tricyclic isomer (254). The cyclobutene ring of (254) was fragmented via ozonolysis to the crystalline ozonide (255), photolytic cleavage[134] then gave the 3-aza-1-dethiapenem derivatives (256) and (257) in 32% and 12% yield respectively. These were not stable enough for characterisation, and so (256) was converted into the corresponding 3-aza-1-dethiapenam (258) by catalytic hydrogenation.

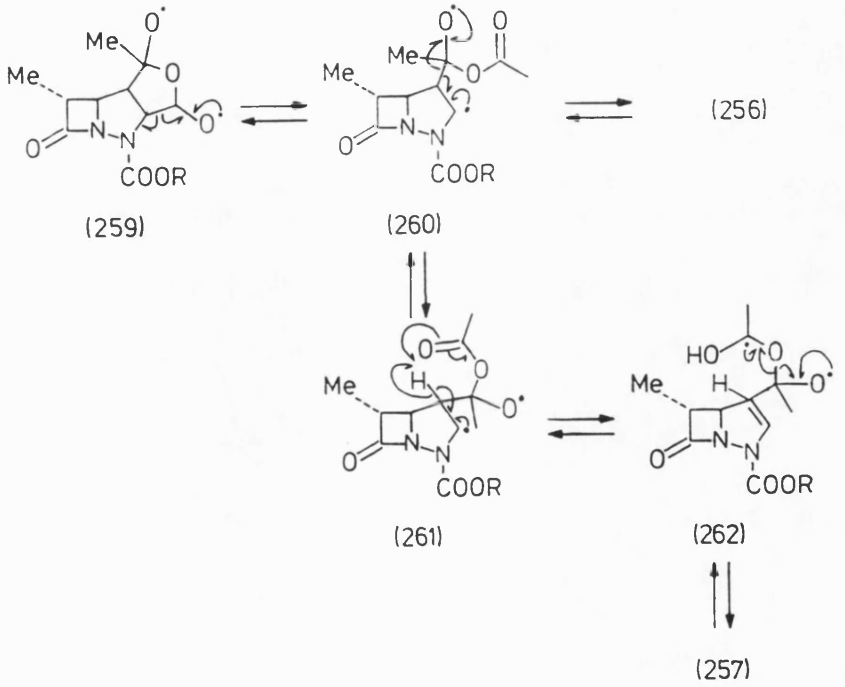


SCHEME 61.

a) hν: b) $CH_3CH=C=O$: c) hν: d) O_3: e) hν: f) H_2, Pd/C.

It is thought that the mechanism of photolytic cleavage of the ozonide (255) is as shown in Scheme 62. It is assumed that the photoexcited ozonide undergoes homolytic O-O bond

cleavage to give the diradical (259) which collapses via diradical (260) to the 3-aza-1-thiapenem (256). Diradical (260) may be in conformational equilibrium with (261), in which intramolecular hydrogen transfer can occur via a 6-membered transition state, leading to diradical (262). Fragmentation of diradical (262) leads to the 1-acetyl-3-aza-1-dethiapenems (257).



SCHEME 62.

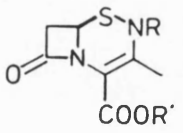
1.3 Summary

In this chapter, the recent syntheses of hetero-analogues of the β-lactam antibiotics have been reviewed. In the following chapter, approaches towards 2-aza-1-thia analogues of cephalosporins and penicillins will be discussed.

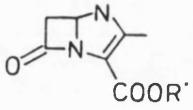
2 DISCUSSION.

2.1 Introduction.

The aim of this project was the synthesis of some new heterocyclic analogues of penicillins and cephalosporins. It has already been seen that a large number of such analogues have already been synthesised, however 2-aza-1-thia analogues have received little attention, the only report of such compounds in the literature being the Hoechst synthesis of the 7-unsubstituted 2-aza-1-thiacephem (263).[135] This compound was used as an intermediate in the synthesis of azapenems (264): desulphurisation of (263) with triphenylphosphine in acetonitrile gave the azapenem (264). This methodology is covered in a patent.[136]



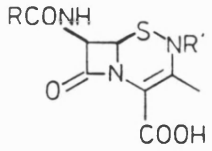
(263)



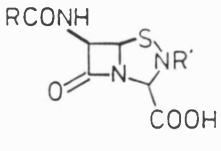
(264)

The Hoechst compounds (263) lack a 7β-acylamido side chain, one of the structural requirements for activity. The free acid is also required for activity; attempts by Hoechst workers to deprotect both (263) and (264) were unsuccessful.

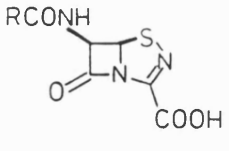
The first target in this programme was therefore the synthesis of 7β-acylamido-4-carboxy-2-aza-1-thiacephems (265). Synthesis of the corresponding penam and penem derivatives (266) and (267) was also to be studied, although it was anticipated that the 2-aza-1-thiapenems (267) may prove too unstable for isolation and characterisation. There are no reports of 2-aza-1-thiapenam or -penem derivatives in the literature.



(265)



(266)



(267)

Before considering the methodology which was proposed for the synthesis of these compounds, let us first consider what is known regarding structure-activity relationships of β-lactam antibiotics, and how these target systems fit these criteria.

There are several review articles[137] which provide an excellent survey of research on the mechanism of action and effect of structural modifications on the activity of β-lactam antibiotics. The penicillins and cephalosporins are thought to act by irreversible acylation of the bacterial transpeptidase responsible for the final cross-linking step in constructing the 3-dimensional network of the bacterial cell wall. Changes in osmotic pressure then cause the rupture of the weakened cell wall.

It is thought that in order to satisfy the requirements for biological activity, a β-lactam antibiotic must possess a combination of several factors, namely; 3D characteristics to enable recognition by the enzyme; and chemical reactivity of the β-lactam bond. These aspects are covered in a recent paper by Cohen.[138]

The requirements for antibacterial activity in penicillin and cephalosporin derivatives are:

a) an amido function α to the β-lactam carbonyl group,

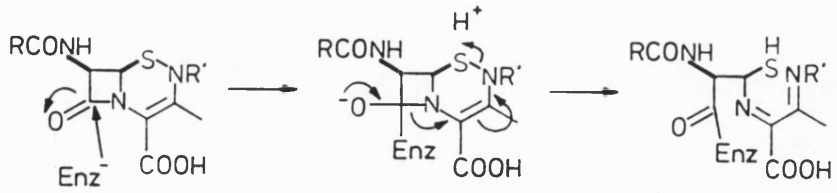
b) a free carboxyl group α to the β-lactam nitrogen atom,

c) **cis** stereochemistry of the amido function relative to the sulphur atom,

and d) a reactive β-lactam bond, activated by strain or electronic factors.

In penicillins the amide linkage is destabilised because the fused 4,5 ring system results in a pyramidal geometry of the β-lactam nitrogen atom, and thus reduces the usual delocalisation of the unshared electron pair on nitrogen into the amide bond. In cephalosporins the activity is increased due to the Δ^3 double bond. A conjugative interaction of the unshared electron pair on nitrogen with the double bond is in competition with the usual stabilisation of the amide bond.

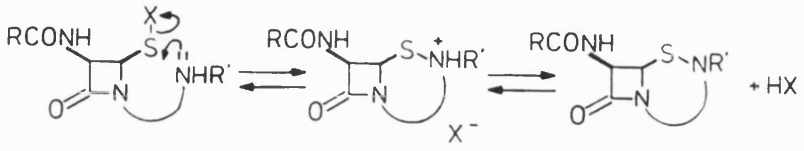
The proposed syntheses would lead to targets satisfying the structural requirements a) to d) above, and which would have unique strain and electronic characteristics. Let us now consider the reactivity of these systems: attack by the transpeptidase enzyme at the β-lactam carbonyl of the 2-aza -1-thiacephem system would be expected to take place readily via the mechanism outlined in Scheme 63.



SCHEME 63.

It was envisaged that the syntheses of all three targets would involve a similar cyclisation step to that employed by

Johnson & Ross in their synthesis of (263). This involves attack of a nitrogen nucleophile at sulphur and expulsion of a good leaving group X. (Scheme 64). Major difficulties to be anticipated could arise from participation of the amide side chain.

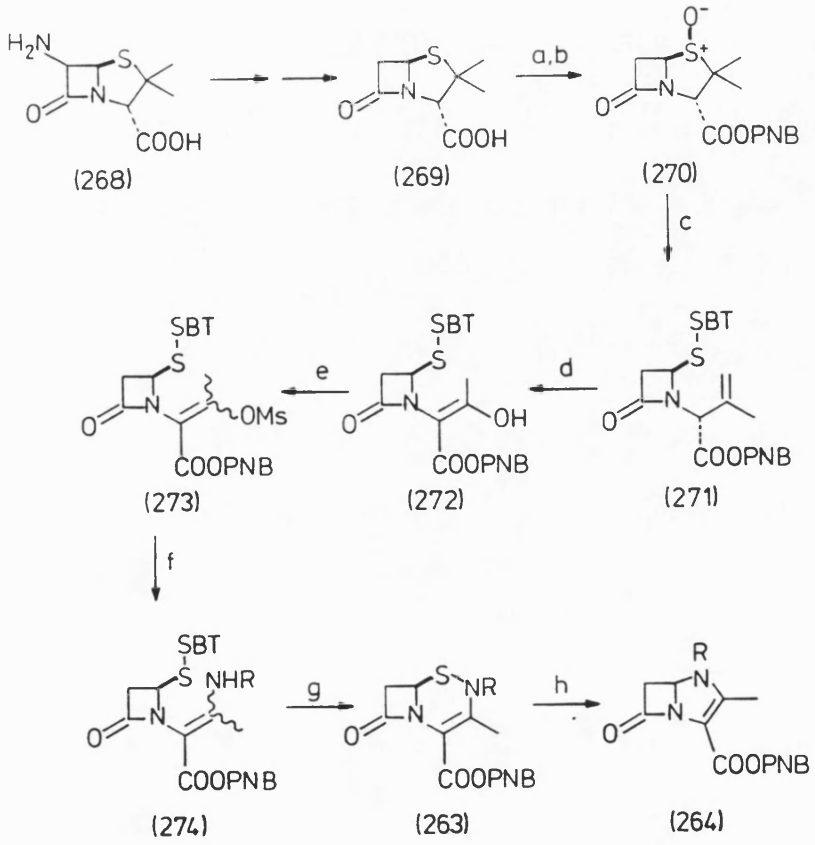


SCHEME 64.

Johnson & Ross employed the S-benzothiazolyl group as the group X, and used a silver salt as a thiophilic agent, thus assisting the expulsion of the leaving group.

It is appropriate to describe the Hoechst synthesis of the 7-unsubstituted 2-aza-1-thiacephem in detail, as this methodology formed a starting point for our approaches to the 7-substituted derivatives. (Scheme 65). Starting from 6-aminopenicillanic acid (268), the vinyl mesylate (273) was synthesised by the method of Beels et al.[139] Conversion of 6-APA into penicillanic acid (269) was effected via 6-bromopenicillanic acid using standard methodology. Penicillanic acid was reacted with p-nitrobenzyl bromide, giving the ester; then converted into the β-sulphoxide (270) by treatment with m-chloroperbenzoic acid. This was converted into the disulphide (271) by the method of Kamiya et al.[140] The sulphoxide (270) was heated under reflux in toluene with 2-mercaptobenzothiazole for 4 hours, yielding (271) as a crystalline solid. This was treated with ozonised

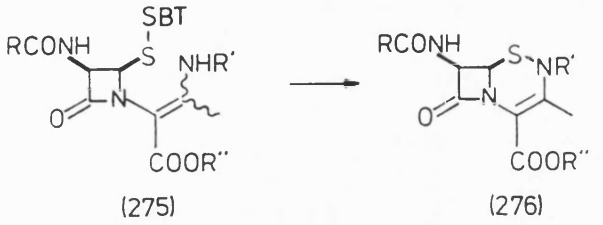
oxygen in dichloromethane at -78°C to give the enol (272); reaction with mesyl chloride and triethylamine in dichloromethane gave the mesylate (273) as a mixture of geometrical isomers. Treatment of the mesylate with an amine in the presence of a strong tertiary amine base yielded the enamino ester (274). Cyclisation of (274) was carried out in the presence of finely divided silver acetate, giving the 2-aza-1-thiacephem (263) in 62% yield. This was desulphurised using triphenylphosphine in acetonitrile at room temp.



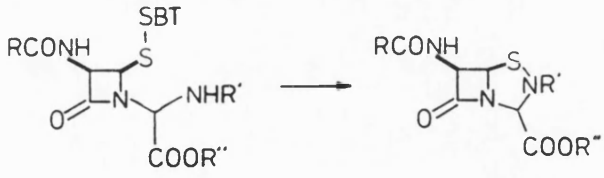
SCHEME 65.

a) PNBBr, $NaHCO_3$, DMF: b) mCPBA, $CHCl_3$: c) HSBT, $PhCH_3$: d) O_3, CH_2Cl_2: e) MsCl, Et_3N, CH_2Cl_2: f)RNH_2, Et_3N: g) C_6H_6, AgOAc: h) PPh_3, MeCN.

It was planned that the synthesis of the 7β-acylamido derivative (276) would proceed via a similar route, involving cyclisation of the enamino ester (275). The proposed synthesis of this intermediate would involve similar methodology to that already described; use of a penicillin as starting material would provide the desired 7-acylamido function, and with the correct stereochemistry.

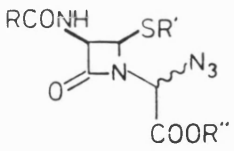


(275) (276)

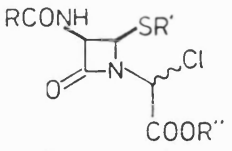


(277) (278)

An analogous approach to the 2-aza-1-thiapenams (278) was also proposed, involving cyclisation of the N-(2'-(2'-amino)-acetate)azetidin-2-one (277). Compounds of this type have not been reported, although azides (279) are known.[141] Possible precursors to (277) included the corresponding azide or the corresponding chloride (280). Examples of similar chlorides are known;[142] these have been used as intermediates in various syntheses of hetero-analogues of penicillins and cephalosporins, and are the precursors in the synthesis of the azide (279). However there are no reports of the 4-dithiobenzothiazolyl compounds (280, R'=SBT) in the literature.

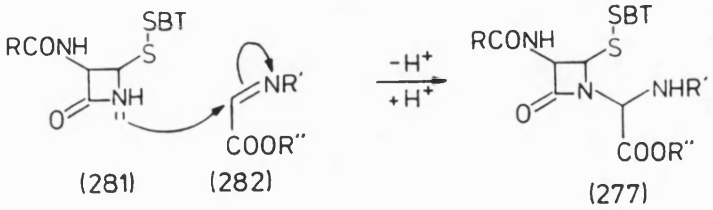


(279)

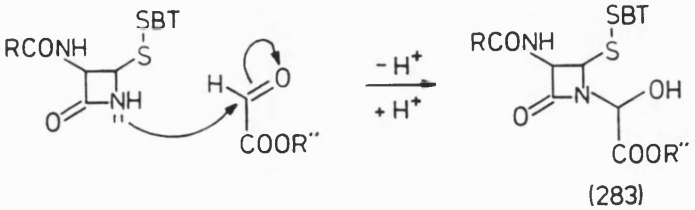


(280)

An alternative approach to 2-aza-1-thiapenams (278) was also proposed. This involved nucleophilic attack by the azetidinone nitrogen of (281) at the imido function of the glyoxylic iminoester (282), which could produce either the N-(2'-(2'-aminoacetate))azetidin-2-one (277) or cyclise spontaneously to the 2-aza-1-thiapenam. This strategy is similar to the well known reaction of azetidinones with glyoxylic esters to give N-(2'-(2'-hydroxy)acetate) derivatives of azetidinones (283).[143]

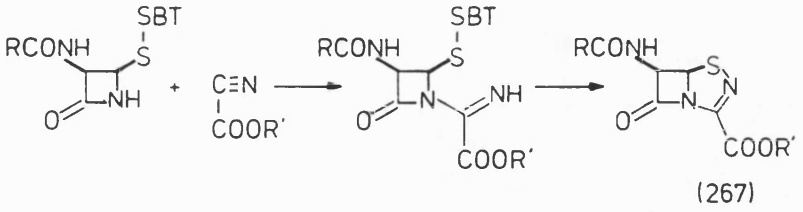


(281) (282) (277)



(283)

The proposed synthesis of 2-aza-1-thiapenems (267) involved nucleophilic attack by the azetidinone nitrogen at the cyano group of a cyanoformic ester, as illustrated in Scheme 66.



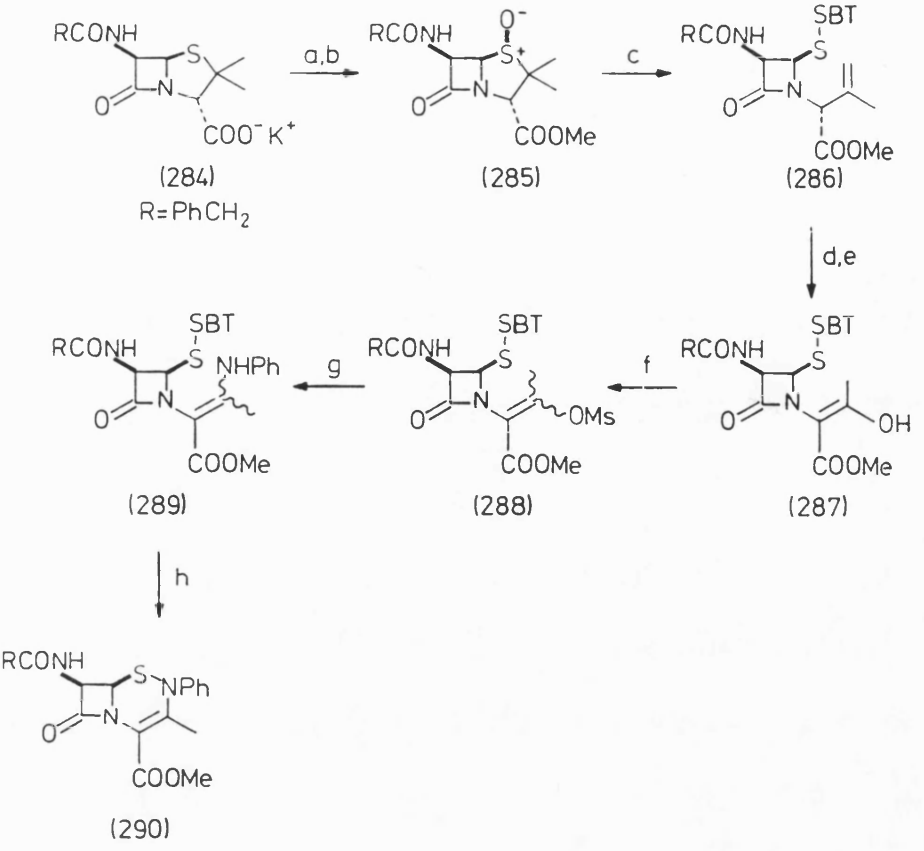
(267)

SCHEME 66.

2.2 Synthesis of 2-aza-1-thiacephems.

It was decided that the first compound to be synthesised should be methyl(2-phenyl 7β-phenylacetamido 2-aza-1-thiacephalosporanate) (290). The methyl ester was chosen to minimise steric effects, and to simplify interpretation of spectra. The synthesis of this compound is illustrated in Scheme 67.

Penicillin G (284) was chosen as starting material, as it possesses the **5R,6R** stereochemistry required in the final product, and is inexpensive and readily available. This was converted into the methyl ester using methyl iodide in N,N-dimethylformamide. Treatment of the methyl ester with m-chloroperbenzoic acid in chloroform gave the β-sulphoxide (285), as interaction between the 6β-amido function and the oxidising agent directs the reagent to the β-face[144] (reagent approach control). It is well known that the stereochemistry of oxidation of penams depends upon the nature of the side chain R'.[144,145] For example, in 6β-phthalimido penicillins, oxidation with m-chloroperbenzoic acid gives the α-sulphoxide as the predominant product: in this case attack occurs at the less hindered face.

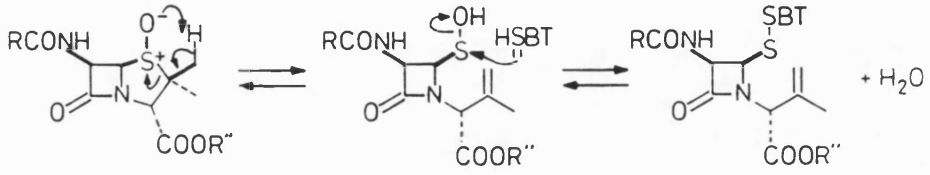


SCHEME 67.

a) MeI, DMF: b) mCPBA, $CHCl_3$, 0°C: c) $PhCH_3$, HSBT, reflux: d) O_3, CH_2Cl_2, -78°C: e) Me_2S: f) MsCl, CH_2Cl_2, -15°C: g) RNH_2, Et_3N, CH_2Cl_2, -15°C: h) C_6H_6, AgOAc, reflux.

Cleavage of the thiazolidine ring of the penicillin sulphoxide (285) was accomplished via the known thermal sigmatropic rearrangement to form a sulphenic acid[146]. These are unstable intermediates, but can be trapped either intramolecularly or intermolecularly by several methods.[147] In this case the method of Kamiya et al. was used: the sulphoxide (285) was heated in toluene under reflux with 2-mercaptobenzothiazole for 2 hours, giving the 4-dithiobenzothiazolyl azetidinone (286) in 61% yield.

The mechanism of this transformation is shown in Scheme 68. Formation of the sulphenic acid is thought to involve a symmetry-allowed sigmatropic [2,3] shift. The sulphenic acid is then trapped by nucleophilic attack by 2-mercaptobenzothiazole.

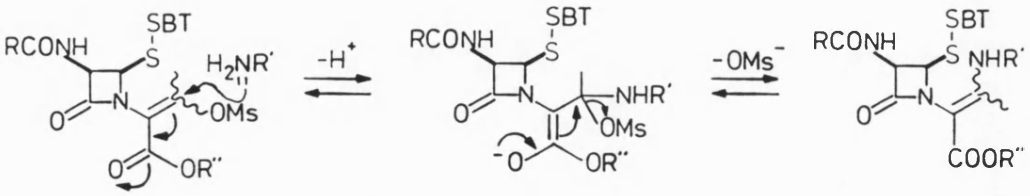


SCHEME 68.

Ozonolysis of the azetidinone (286) was carried out at -78° C in dichloromethane. A blue colouration due to an excess of unreacted ozone in the solution indicated when the reaction was complete, and the ozonide was worked up by addition of dimethyl sulphide. The crude product was purified by chromatography on silica, giving the enol (287) in 60% yield. Ozonolysis of (286) could, in theory, give rise to a mixture of the keto form, and **E** and **Z** isomers of the enol form. The geometrical isomers of the enol are theoretically interconvertible via the keto form; the enol form should be thermodynamically more favourable, due to conjugation with the ester group. Indeed, in this case the keto form was not detected in the nmr spectrum. One would also expect that the **E** enol would predominate, due to hydrogen-bonding between the ester group and the enolic -OH. In the case of the enol (287) it was not possible to prove this conclusively on the basis of its 60MHz nmr spectrum. However, an analogous enol was later prepared, and the high resolution nuclear magnetic resonance spectrum of this compound indicated a predominance of the **E** isomer.[148]

The Hoechst workers had found that conversion of the enol (272) into the desired enamine (274) required the conversion of the enolic -OH into a better leaving group. In the Hoechst route to the 7-unsubstituted 2-aza-1-thiacephem (263), mesylate was the leaving group of choice; for this reason it was chosen as the first candidate in our studies. Treatment of the enol (287) with 1 equivalent mesyl chloride and 1 equivalent triethylamine in dichloromethane at -15°C, followed by chromatography on silica gave the mesylate (288) in 45% yield as a mixture of geometrical isomers. This was treated with 1 equivalent of aniline and 1 equivalent of triethylamine in dichloromethane at 0°C. Subsequent chromatography on silica gave the enamine (289) in 35% yield as a mixture of geometrical isomers.

The conversion of the mesylate (288) to the enamine (289) is thought to involve a Michael-type addition of aniline to the mesylate, followed by elimination of methanesulphonic acid, as shown in Scheme 69.



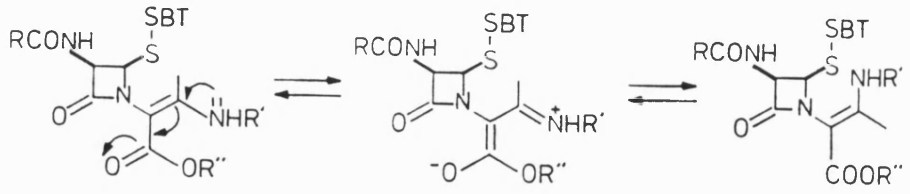
SCHEME 69.

The enamine (289) was cyclised to (290) by heating in benzene under reflux in the presence of finely divided silver acetate. It was found that this step requires the use of scrupulously dry benzene, vigorous stirring, and high dilution to discourage intermolecular attack. Even once these

precautions have been taken, the cyclisation reaction proceeds only slowly and in low yield (13%). It is thought that this is due to a combination of several factors: firstly the thermal instability of the reactive 2-aza-1-thiacephem system. Secondly, the enamine (289) exists as a mixture of **E** and **Z** isomers. It is the **Z** isomer which has the required geometry for cyclisation, but unfortunately the **E** isomer is likely to be the major isomer, as it is stabilised by hydrogen bonding between the ester group and the amine group.

The progress of the cyclisation reaction was monitored by thin layer chromatography using 60% ethyl acetate in petroleum ether. The enamine showed as two spots with Rf 0.30 and 0.27, which were in fact so close together that they represented a "figure 8" shape on the plate. During the reaction the more polar of these disappeared more rapidly than the other, accompanied by the formation of a new spot at Rf 0.54. All of the more polar isomer reacted over a period of 1 hr, but reaction of the other isomer was incomplete. It can be inferred that the more polar spot by TLC is due to the **Z** isomer, which would be expected to undergo cyclisation with greater ease.

In theory the **E** and **Z** isomers of (289) should be in equilibrium with the intermediate (291), as shown in Scheme 70. Intermediate (291) allows free rotation around the 2'C-3'C bond, thereby enabling interconversion of the **Z** and **E** isomers. However, it seems that the energy barrier involved in this process results in very slow interconversion, if at all.



(291)

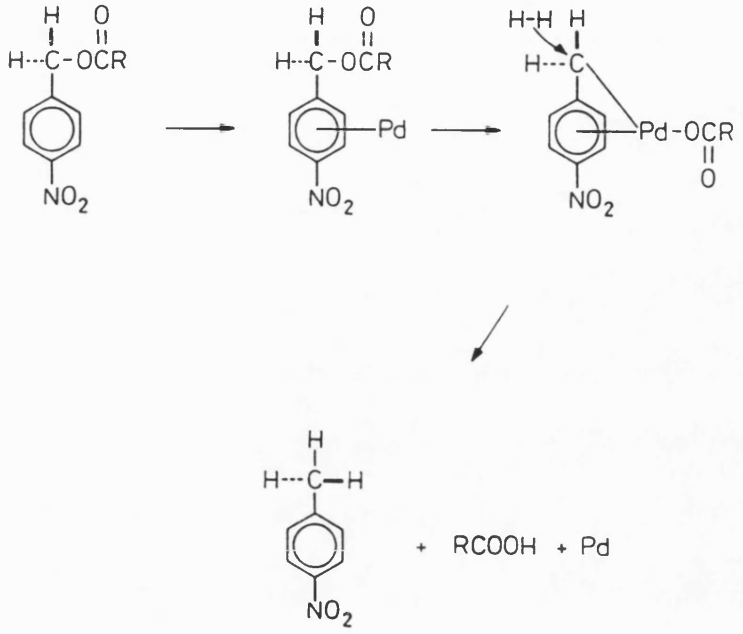
SCHEME 70.

No attempt was made to deprotect the methyl ester (290), partly due to a lack of material, and partly because finding a method of hydrolysing the ester without cleavage of the labile β-lactam ring would prove rather difficult. However, as a result of the synthesis of the 7β-acylamido-2-aza-1-thiacephem (290) we had satisfied ourselves that the presence of the 7β-acylamido side chain did not interfere with the Hoechst methodology. The next step in our investigations was therefore the synthesis of an analogous 2-aza-1-thiacephem having a different acid protecting group which could be removed in the presence of a reactive β-lactam ring.

The p-nitrobenzyl acid protecting group was chosen for use in the next synthesis of 7β-substituted 2-aza-1-thiacephems, as it is easily removed by palladium catalysed hydrogenolysis;[149] and removal of the p-nitrobenzyl group by this method can be carried out in the presence of β-lactam rings.[150]

There have been several studies on the mechanism of hydrogenolysis of benzyl derivatives, and it has been found that the reaction shows substantial stereospecificity.[151] Whether the reaction proceeds with retention or inversion of stereochemistry depends upon the catalyst used. In the case of palladium, the reaction proceeds with inversion, and Khan

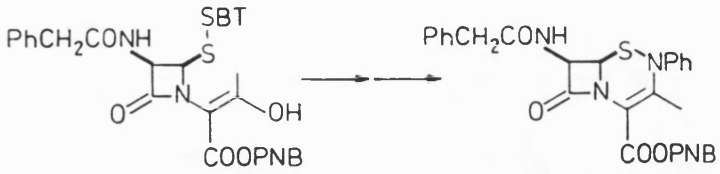
et al. have suggested the mechanism shown in Scheme 71. In the case of p-nitrobenzyl esters, R'=R"=H and X=OCOR; and as such esters are achiral, these stereochemical considerations have little consequence in this case.



SCHEME 71.

The synthesis of the p-nitrobenzyl protected 2-aza-1-thia cephem (293) involved the preparation of the intermediate (292) by a route analogous to the preparation of the corresponding methyl analogue (287). The intermediate (292) was treated with mesyl chloride and triethylamine, giving a mixture of the mesylate and unreacted starting material. Separation of these two compounds by chromatographic methods proved to be very difficult. The reaction was repeated using mesyl chloride in pyridine, but in this case cleavage of the β-lactam ring occurred. However, treatment of the mixture with an amine in the presence of triethylamine, followed by

cyclisation in benzene under reflux and in the presence of silver acetate allowed the isolation of a small quantity of p-nitrobenzyl(2-phenyl 7β-phenylacetamido-2-aza-1-thia-cephalosporanate) (293).

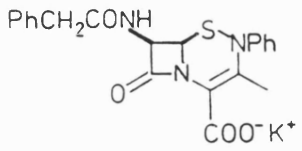


(292) (293)

p-Nitrobenzyl(2-phenyl 7β-phenylacetamido-2-aza-1-thia-cephalosporanate) (293) was treated with hydrogen over a palladium catalyst in a Paar apparatus at 50psi in the presence of potassium hydrogen carbonate, giving the deprotected 2-aza-1-thia cephem (294) in 14% yield.

The spectroscopic data obtained for this compound is as follows. In the infra-red spectrum three carbonyl stretching frequencies were observed at 1785, 1710 and 1660cm^{-1}, corresponding to the β-lactam carbonyl, the amide carbonyl and the carboxylate group respectively. The nuclear magnetic resonance spectrum showed the C-6 proton as a doublet at 5.33ppm, and the C-7 proton as a doublet of doublets at 5.96ppm. A singlet was observed at 1.63ppm, which was assigned as the 3-methyl group. The CH_2 protons of the acylamido side chain appeared as a quartet at 4.81ppm, and integration of the aromatic signals indicated the presence of 10 aromatic protons. A mass spectrum was obtained using negative ion Fast Atom Bombardment (FAB). This showed a significant peak at m/z 365, corresponding to the loss of CO_2 from the molecular ion, together with a number of fragment ions.

The above data indicates that the product has the structure (294).



(294)

ORGANISM	MIC µg/ml
Str. pyogenes 77A	12.5
Str. pyogenes 308A	12.5
Str. faecium D	>50
Staph. aureus SG 511	50
Staph. aureus 285	50
Bac. subt. ATCC 6633	12.5
Ps. aerug. NCTC 10701	>50
Ps. aerug. 1771	>50
E. coli 055	>50
E. coli DC 0	>50
Kl aerog. 1082E	>50
Ent. cloacae P99	>50

TABLE 2.

The deprotected 2-aza-1-thiacephem (294) was tested for antimicrobial activity. The minimum inhibitory concentration (MIC) against a variety of microorganisms is given in Table 2, showing that this compound exhibits weak antibacterial activity against some Gram +ve organisms.

An analogue of the intermediate (292) having a 7β-phenoxy-acetamido side chain was prepared using the methodology described earlier. Reaction of this analogue (295) with mesyl chloride and triethylamine was attempted, and again the reaction did not proceed to completion.

(295) (296)

Various alternative methods for the conversion of the enol (295) to enamines of the type (296) were studied. It was thought that the keto form of the compound (295) might react with an amine to form an imine, which could then tautomerise to the enamine. A solution of the enol (295) in dichloromethane was treated with aniline in the presence of a drying agent ($MgSO_4$). No reaction was observed. The procedure was repeated using aminoacetonitrile instead of aniline. Again no reaction was observed.

The 250MHz nmr spectrum of the compound (295) shows the compound to be exclusively one geometrical isomer of the enol form, although it is possible that the other isomer of the enol, and the keto form are present only in very small quantities not detectable by nmr. However the above experiments seem to indicate that the amount of keto form present is negligible.

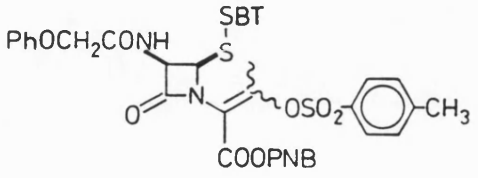
One geometrical isomer of the enol (295) is expected to be thermodyamically more favourable due to the possibility of hydrogen bonding between the ester group and the enolic -OH group. Hence detection of exclusively one isomer in the nmr spectrum is not surprising, and it is reasonable to assume that this isomer contains the -OH and ester groups **cis** to one another. In the IR spectrum the -OH stretch occurs at 2990cm^{-1}, indicating intramolecular hydrogen bonding; and

the ester C=O stretch occurs at 1730cm^{-1}, compared with 1745cm^{-1} in the disulphide. This shift to lower wavenumber indicates that the ester group is involved in hydrogen bonding.

The reaction of the enol (295) with aniline in the presence of diethylazodicarboxylate and triphenylphosphine was also studied. The procedure was carried out as described by Mitsunobu.[152] No reaction was observed.

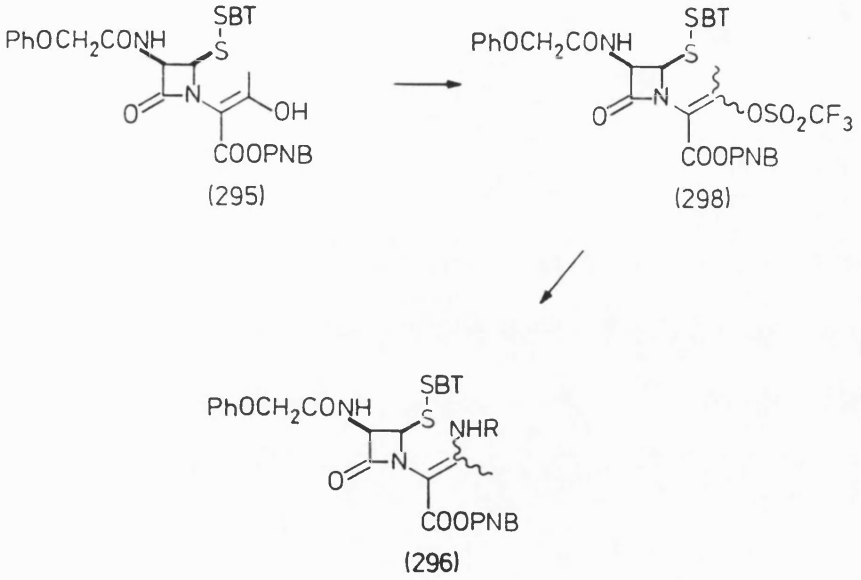
The use of alternative leaving groups was also studied. In an attempt to prepare the corresponding acetate a solution of enol (295) in dichloromethane was treated with acetic anhydride and a base. No reaction was observed, and starting material was recovered.

Treatment of the enol with tosyl chloride and triethylamine produced a mixture of the tosylate (297) (29%) and starting material. It was possible to separate the tosylate from the starting material by rapid flash chromatography, but some decomposition of the tosylate took place on the silica. In an attempt to improve the yield of this reaction the use of sterically hindered non-nucleophilic bases was studied, and although the use of N,N-diisopropylethylamine caused a slight improvement in yield (37%), the reaction still did not go to completion.

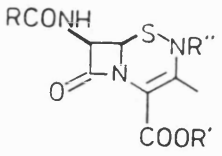


(297)

Finally it was found that conversion of the enol to the triflate (298) using triflic anhydride and N,N-diisopropyl-ethylamine; followed by immediate reaction with the desired amine <u>in situ</u> gave an improved yield of enamine (296) (50-60% over two steps). In one instance an attempt was made to isolate the triflate, and this was achieved in 69% yield, although some decomposition during chromatography was noted.



Several examples of the 2-aza-1-thiacephem system were synthesised. These are summarised in Table 3. Deprotection of the 2-aza-1-thiacephems (293), (300), and (301) was attempted, although only (293) yielded enough product of sufficient purity for characterisation. In the other cases the products isolated showed no azetidinone carbonyl stretching frequency in the IR spectrum. It was inferred that the 2-aza-1-thiacephem system is not sufficiently stable to withstand the conditions needed to effect hydrogenolysis of the p-nitrobenzyl ester.



	R	R'	R''
(290)	$PhCH_2$	Me	Ph
(293)	$PhCH_2$	pNB	Ph
(299)	$PhCH_2$	pNB	Me
(300)	$PhOCH_2$	pNB	Ph
(301)	$PhOCH_2$	pNB	CH_2CN

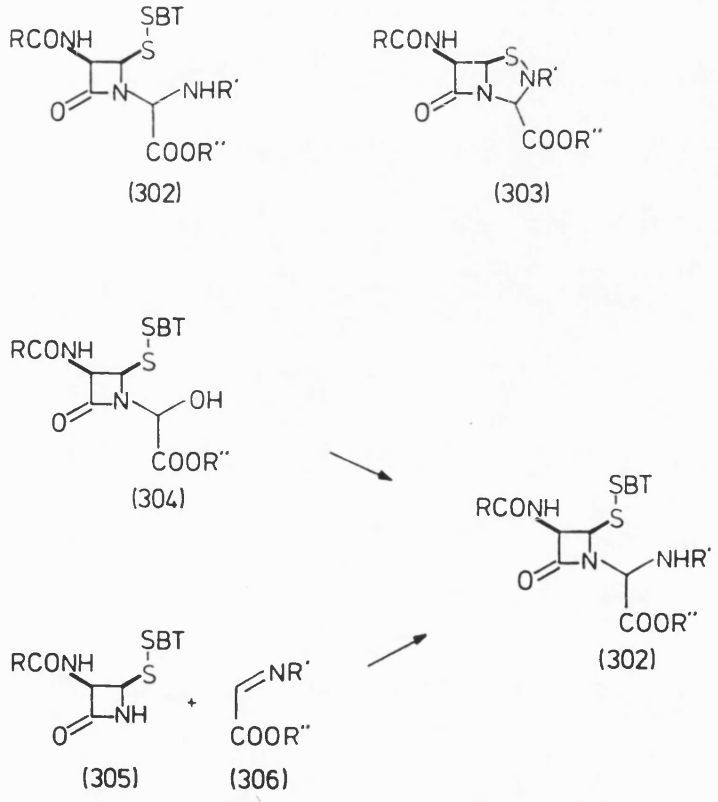
TABLE 3.

It was thought that 2-aza-1-thiacephems having R"=COPh or R"=OCOPh might prove to be more stable under the conditions required for deprotection of the ester group. The preparation of the precursors required for the synthesis of these compounds was attempted: the triflate (298) was treated with benzamide in the presence of base, and with phenyl carbamate in the presence of base. In both cases no reaction was observed, indicating that neither benzamide nor phenyl carbamate are sufficiently nucleophilic to cause displacement of the triflate group.

In conclusion, therefore, the first 7β-acylamino-2-aza-1-thiacephalosporanate has been synthesised, and low level antibiotic activity against Gram positive organisms has been demonstrated.

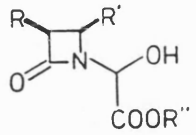
2.3 Synthesis of 2-aza-1-thiapenams.

The proposed route to the 2-aza-1-thiapenams (303) involved cyclisation of an intermediate having the structure (302) by a procedure analogous to that already described in the synthesis of 2-aza-1-thiacephems. Two approaches to intermediates of type (302) were proposed: firstly conversion of 1-(1-azetidin-2-one)-yl-1-hydroxyacetate precursors (304) into the desired intermediates; and reaction of N-unsubstituted azetidinones (305) with an imine derived from a glyoxylic ester (306).



The approach involving the precursor (304) will be discussed first. An extensive search of Chemical Abstracts revealed no examples of azetidinone derivatives having both a dithiobenzothiazolyl group at the 4-position and a 1-hydroxyacetate moiety attached to the 1-position (307, R'=SSBT).

However, in the literature there are a number of examples of similar compounds having other substituents in the 4-position. Some examples of these are given in Table 4.



(307)

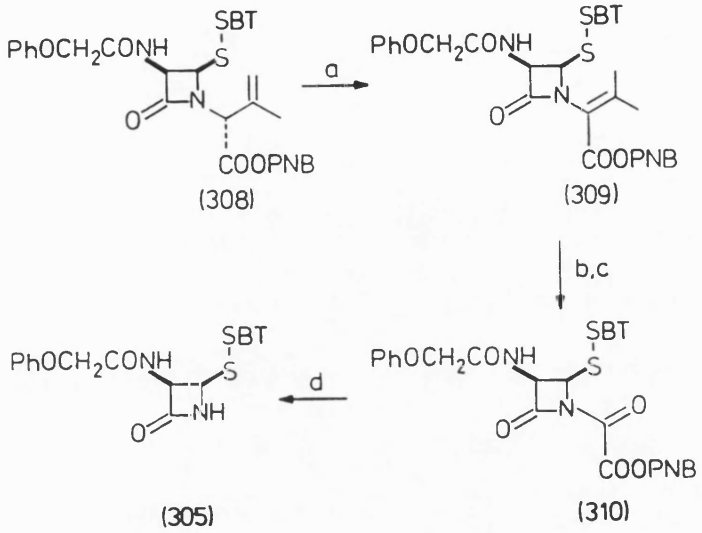
R	R'	R''	ref.
$PhOCH_2CONH$	SCOMe	Me	153
$PhOCH_2CONH$	SCOMe	pNB	153
$PhOCH_2CONH$	SCOMe	CH_2CCl_3	153
$PhOCH_2CONH$	$SCH=CHCO_2Et$	pNB	153
$PhOCH_2CONH$	$SCH=CHCO_2Et$	tBu	153
$PhOCH_2CONH$	$CH=CH_2$	Bz	154
Ph_3CNH	SMe	tBu	141
Ph_3CNH	$SCH_2C=CH$	tBu	141

TABLE 4.

The examples in Table 4 were all prepared via reaction of the appropriate N-unsubstituted azetidinone with the appropriate glyoxylic ester, usually in the form of a hydrate or hemiacetal. Water was removed from the reaction by the use of either molecular sieves or a Dean-Stark head.

A considerable quantity of the azetidinone (305) was prepared; as this was required for use both in the preparation of the 1-hydroxyacetate (304), and as an intermediate in the work described later in this thesis.

The unsubstituted azetidinone (305) was used by Woodward as an intermediate in his early penem syntheses;[153] he synthesised this intermediate in two steps from the disulphide (309), which is easily prepared by base-catalysed isomerisation of Kamiya's disulphide (308). The synthesis of the N-unsubstituted azetidinone (305) is outlined in Scheme 72.



SCHEME 72

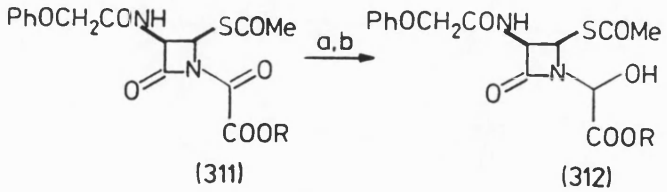
a) Et_3N, CH_2Cl_2: b) O_3, MeOAc, -78°C: c) aq. $NaHSO_4$: d) MeOH/ MeOAc/H_2O, reflux.

A supply of disulphide (308) was already in hand, as it had been prepared for use as an intermediate in the synthesis of the 2-aza-1-thiacephems. Treatment of Kamiya's disulphide (308) with triethylamine in dichloromethane at room temp., followed by washing with aq. citric acid to remove triethylamine, gave the isomeric disulphide (309). The reaction was followed by observing the bathochromic shift of the ester carbonyl stretch as the double bond became conjugated with the ester group. The IR stretching frequencies of the starting material and product are compared in Table 5.

ν max	(308)	(309)
β-lactam C=O	1770	1770
ester C=O	1750	1725
amide C=O	1685	1690

TABLE 5.

Cleavage of the olefinic group in (309) was achieved by passing a stream of ozonised oxygen through a solution of (309) in methyl acetate at -78°C. The excess ozone was removed by a stream of nitrogen, the solution was allowed to warm to 0°C and washed with a cold aqueous solution of sodium bisulphite, effecting reductive hydrolysis of the ozonide. The crude, crystalline product (310) was generally used in the methanolysis reaction without further purification. It is interesting to note that Woodward et al. carried out a diborane reduction of the intermediate (311),[153] giving the compound (312) (Scheme 73), which he did not purify but used crude in the next step. Woodward reports that although this method represented a saving of one synthetic step in the overall route it was found that in most cases the longer route via the N-unsubstituted azetidinone proved more practical.



(311) (312)

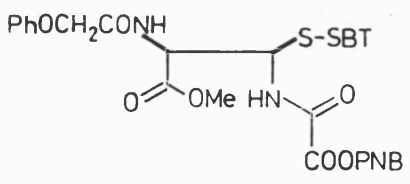
SCHEME 73.

a) B_2H_6, THF, 0°C: b) NH_4Cl aq.

Mild methanolysis of the compound (310) was carried out by treatment with a mixture of aqueous methanol and methyl acetate at reflux for 30 min. Crystallisation of the crude product yielded a mixture of the desired azetidinone (305) and methyl p-nitrobenzyl oxalate. These were separated by flash chromatography, using 20% ethyl acetate/petrol to elute methyl p-nitrobenzyl oxalate, then eluting the azetidinone (305) with ethyl acetate. It is interesting to note that Woodward quotes nmr data for this compound in $CDCl_3/CD_3OD$ solution - in our hands it was found to have only moderate solubility in methanol and did not succeed in obtaining an nmr spectrum in $CDCl_3/CD_3OD$. However, it was found that the azetidinone (305) was reasonably soluble in THF, and an nmr spectrum in deuterated THF was obtained which correlated reasonably well with Woodward's data (allowing for chemical shift differences due to change in solvent). The lack of solubility of (305) in many of the commonly used solvents proved to be an irritating constraint in the choice of solvent used in reactions involving this compound, hence THF was the solvent of choice wherever possible.

A similar two-step removal of the N substituent has also been used in a penicillin degradation by Cooper and Jose.[154]

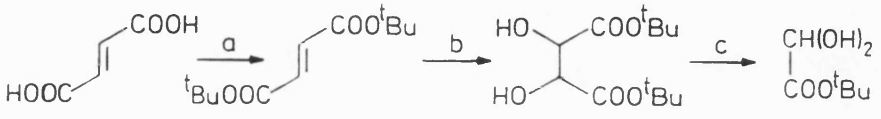
It is noted that methanolysis of the β-lactam amide bond to give (313) is also a possibility. A small amount of a polar impurity was noted during the TLC analysis of the crude reaction mixture, which is likely to have arisen in this way. Also, Woodward has isolated these minor byproducts in the course of his work.



(313)

The next step in this synthetic route involved condensation of the azetidinone (305) with a glyoxylic ester. Glyoxylic esters are not commercially available, so these were synthesised by known routes. In this work t-butyl and p-nitrobenzyl glyoxylates were used.

t-Butyl glyoxylate was prepared in three steps from fumaric acid by a route established by Blake et al.[155] (Scheme 74).



(314) (315) (316) (317)

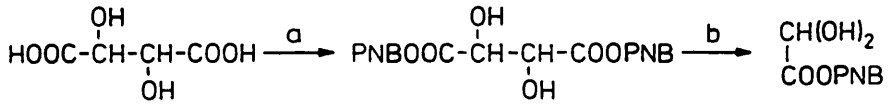
SCHEME 74.

a) isobutylene, conc. H_2SO_4, diglyme: b) aq. $KMnO_4$, ^{t}BuOH: c) $Pb(OAc)_4$, dry benzene.

Fumaric acid (314) was shaken with isobutylene and conc. sulphuric acid in diglyme in a sealed tube. After aqueous work-up di-t-butyl fumarate (315) was isolated in 64% yield. The fumarate was dissolved in t-butanol and oxidised with aqueous potassium permanganate solution, giving di-t-butyl tartrate (316) in 48% yield. The tartrate was converted to t-butyl glyoxylate (317) by oxidative cleavage with lead tetraacetate in dry benzene. It was found that recrystallisation of the lead tetraacetate from acetic acid just prior to use improved the yield of this reaction: after work-up and distillation a 53% yield of t-butyl glyoxylate monohydrate was obtained. The spectral data indicate that the hydrated form predominates - in the nmr the aldehyde proton occurs as a singlet at 9.3ppm, and multiplets occur at 3.64 and 3.80ppm due to the hydrate protons.

An alternative method[156] of preparation of t-butyl glyoxylate involves preparation of the t-butyl ester of methoxyacetic acid, conversion to the α-bromo derivative with N-bromosuccinimide, followed by treatment with aqueous sodium hydrogen carbonate to give t-butyl glyoxylate monohydrate. However, the former method of preparation was used as the intermediates involved are crystalline solids.

p-Nitrobenzyl glyoxylate was prepared[157] in two steps from tartaric acid. (Scheme 75). Tartaric acid was converted into its di-p-nitrobenzyl ester (318) by treatment with p-nitrobenzyl bromide and triethylamine in DMF. The tartrate was subjected to oxidative cleavage using periodic acid in THF, giving p-nitrobenzyl glyoxylate monohydrate (319) in 45% yield over the two steps.



(318) (319)

SCHEME 75.

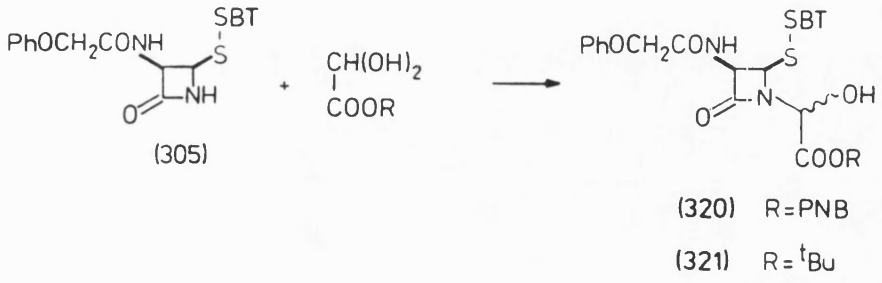
a) PNBBr, Et_3N, DMF: b) H_5IO_6, THF/H_2O.

The azetidinone (305) was condensed with p-nitrobenzyl glyoxylate by heating in benzene under reflux (Scheme 76). During the reaction water was removed by using a Dean-Stark head. Although a moderate yield (approx. 40%) of the desired azetidin-2-one-1-yl hydroxyacetate (320) was obtained by this method, a relatively long reaction time was needed, and as a consequence some decomposition of material was noted. It is thought that this problem is largely due to the poor

solubility of the azetidinone (305) in benzene. A modification of the reaction conditions involving the use of dry THF as solvent and molecular sieves as a method of removing water proved more satisfactory.

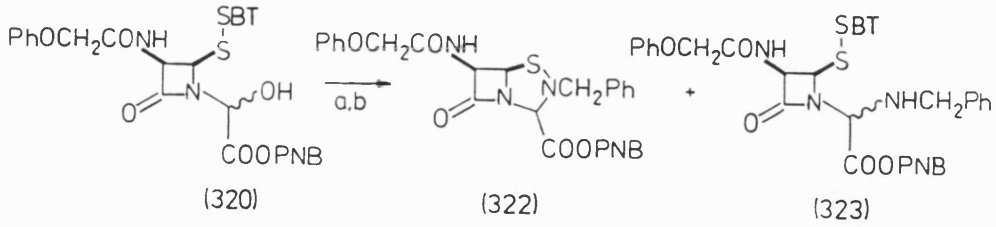
The corresponding t-butyl 1-(1-azetidin-2-one)-yl-1-hydroxy-acetate (321) was prepared in a similar manner from the azetidinone (305) and t-butyl glyoxylate monohydrate.

In both cases a diastereomeric mixture of alcohols was produced, and attempts to separate the diastereomers were unsuccessful.



SCHEME 76.

The diastereomeric mixture (320) was treated with 2,6-lutidine and thionyl chloride at -15°C, a method similar to that used by Pearson et al.[158] (Scheme 77). The product was not isolated but was treated immediately with benzylamine. Subsequent work-up yielded a complex mixture of products which was purified by flash column chromatography, yielding a small amount of a diastereomeric mixture of 1-(1-azetidin-2-one)-yl-1-(benzylamino)acetate (323), together with a small amount of the cyclised product, p-nitrobenzyl [2-benzyl-6β-phenoxyacetamido]-2-aza-penicillanate] (322) as a mixture of diastereomers.



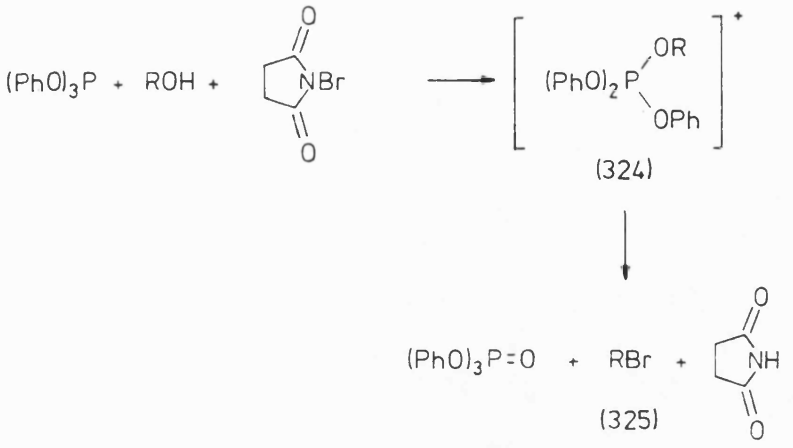
(320) (322) (323)

SCHEME 77.

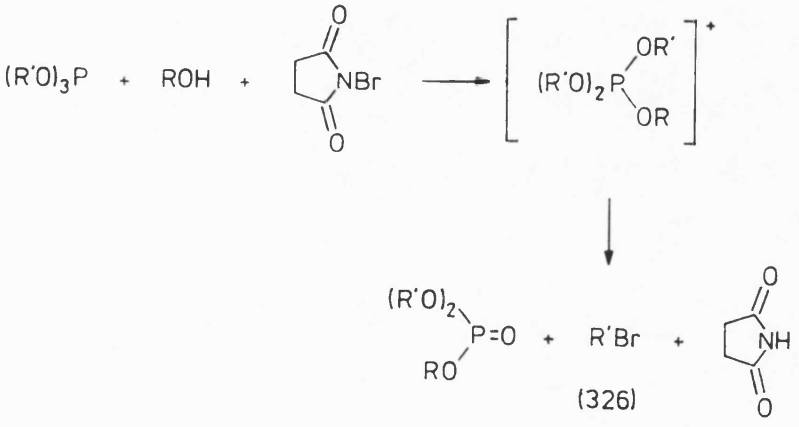
a) $SOCl_2$, 2,6-lutidine: b) $PhCH_2NH_2$.

A similar reaction was carried out on t-butyl 1-(1-azetidin-2-one)-yl-1-hydroxyacetate, which again yielded a complex mixture of products. Attempts to isolate these yielded only compounds not containing a β-lactam ring. It is thought that the bulky t-butyl group causes steric congestion, thereby hindering nucleophilic attack by the amine.

In an attempt to improve the efficiency of the reaction the use of other halogenating agents was investigated. Reaction of the intermediates (320) and (321) with N-chloro-succinimide and triphenylphosphite, according to the procedure of Bose & Lal,[159] was investigated. This method involves the formation of an intermediate phosphonium salt (324); followed by cleavage of the intermediate to give a trialkyl phosphate with concomitant formation of the halide (325). This will be the major course of the reaction when triphenyl phosphite is used. (Scheme 78). However, if a trialkyl phosphite is used, the intermediate is cleaved in an alternate sense, giving the halide (326)[161] (Scheme 79).

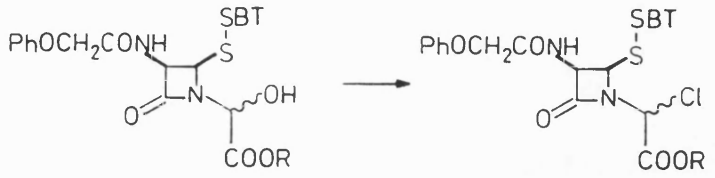


SCHEME 78.



SCHEME 79.

N-bromosuccinimide/triphenylphosphite has been used as a reagent for the conversion of sterols to bromosteroids, a reaction which proceeded rapidly under the mild reaction conditions, giving high yields and high stereospecificity. Therefore the method was chosen as a possible candidate for the conversion of the intermediates (320) and (321) to the corresponding halo-compounds (327).



(320) R=PNB

(321) R=^{t}Bu

(327)

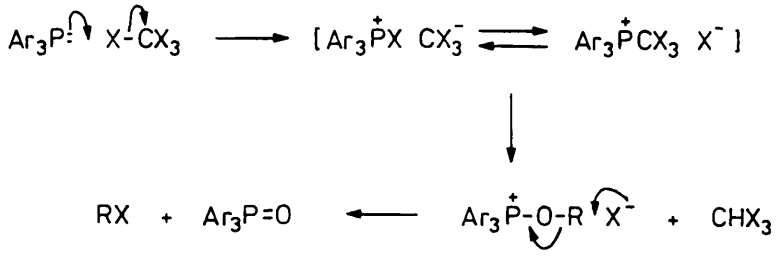
When the intermediates (320) and (321) were treated with N-chlorosuccinimide and triphenylphosphite, a dark brown colour was formed immediately, and no β-lactam containing compounds were observed.

As N-halosuccinimides are known to be efficient oxidising agents for the conversion of alcohols to ketones,[161] it is possible that this was a competing reaction in this case. Alternatively, it is possible that the reagent caused cleavage of the β-lactam ring.

Another mild chlorinating agent for alcohols is cyanuric chloride.[162] This has been used for the conversion of aliphatic alcohols to the corresponding chlorides. A solution of the intermediate (320) in dichloromethane was

treated with cyanuric chloride, but no reaction was observed and starting material was recovered.

The use of carbon tetrachloride and triphenylphosphine as a reagent for chlorination of hydroxyl groups in carbohydrates is well established.[163] It is generally accepted that these reactions proceed via an ionic mechanism as shown in Scheme 80.



SCHEME 80.

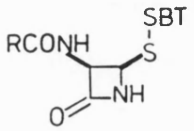
The rate determining step in this reaction seems to be the abstraction of "Cl^+" by triphenylphosphine, and the rate of reaction ought to be increased if a better leaving group than $Cl_3C:^-$ were involved. This consideration led to a modification of this method involving the use of hexachloroacetone/triphenylphosphine.[164] This modification was used in an attempt to effect the conversion of (320) to (327), but no reaction was observed and starting material was recovered.

Other methods of activating the 1-(1-azetidin-2-one)-yl-1-hydroxyacetate (320) towards nucleophilic attack were investigated. The reaction with an amine in the presence of diethylazodicarboxylate and triphenylphosphine (Mitsunobu reaction)[152] was studied. A complex mixture of products was observed. These were purified by flash column

chromatography and isolated, although none of these proved to be an azetidinone derivative.

Conversion of the hydroxyl group into a mesylate or a tosylate was attempted, but no reaction was observed and starting material was isolated. The 1-(1-azetidin-2-one)-yl-1-hydroxyacetate was treated with trifluoromethane-sulphonic anhydride and N,N-diisopropylethylamine. It was expected that the corresponding triflate would be a very reactive intermediate, therefore no attempt was made to isolate it and it was treated with benzylamine in situ. A mixture of very polar products was produced which were thought to arise from cleavage of the β-lactam ring.

At this point an alternative approach to the 2-aza-1-thiapenams was investigated, namely the reaction of the N-unsubstituted azetidinone (305) with the imine of a glyoxylic ester (306).



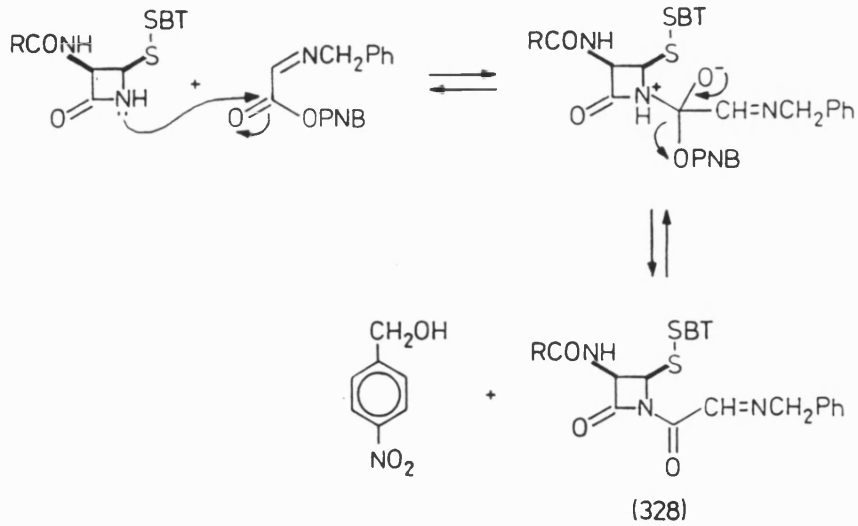
(305)

(306)

The imine (306) was prepared by condensation of a glyoxylic ester with an appropriate amine in a procedure similar to that of Hakimelahi:[165] a solution of the glyoxylic ester in dichloromethane was added to a stirred solution of the amine in dichloromethane at 0°C, in the presence of a drying agent such as magnesium sulphate or molecular sieves. It was found that the crude imine could be purified by rapid flash chromatography using silica gel which had previously been deactivated by shaking with 10% water. Examples of the

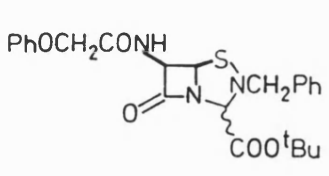
imine (306) where R"=p-nitrobenzyl or t-butyl, and R'= benzyl were prepared from p-nitrobenzyl and t-butyl glyoxylate respectively. The synthesis of these glyoxylic esters has already been described.

The reaction of the azetidinone (305) with p-nitrobenzyl (1-benzyliminoacetate) was carried out in tetrahydrofuran at reflux under a nitrogen atmosphere. A complex mixture of products was obtained, however an infrared spectrum of the crude mixture showed the absence of any azetidinonyl carbonyl stretching frequency and furthermore a significant quantity of p-nitrobenzyl alcohol was isolated. These observations indicate that nucleophilic attack by the azetidinone nitrogen took place at the ester moiety of (306) and not at the imine function as hoped. (Scheme 81). The azetidinonyl moiety (328) thus produced would be of limited stability and could be expected to decompose by any of several mechanisms.

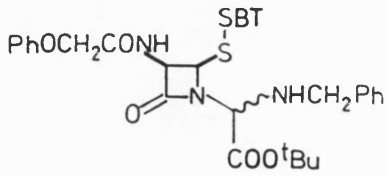


SCHEME 81.

The reaction of the azetidinone (305) with t-butyl (1-benzyliminoacetate) was carried out in a similar way, and it was hoped that in this case the steric effect of the t-butyl group would hinder nucleophilic attack at the "wrong" centre and thus encourage formation of the desired product. Indeed, a small quantity of product (ca. 12% yield) was isolated, and from the spectroscopic data for this it was deduced that this product was the 2-aza-1-thiapenam (329) as a mixture of diastereomers. In the infra-red spectrum three carbonyl stretching frequencies were observed at 1765, 1720 and 1690cm^{-1}, corresponding to the β-lactam carbonyl, the ester carbonyl and the amide carbonyl respectively. The nuclear magnetic resonance spectrum showed no resonance due to a benzothiazolyl group, and integration of the aromatic signals indicated the presence of 10 aromatic protons. Two singlets were observed at 4.92 and 4.99 ppm, which were assigned as the 3-α and 3-β protons, indicating that the product was a mixture of diastereomers. Also observed were two doublets at 5.23 and 5.37 ppm, due to the 7-α proton of each diastereoisomer; and two doublets of doublets at 5.15 and 5.26 ppm, due to the 6-α proton of each diastereoisomer. A mass spectrum was obtained using positive ion Fast Atom Bombardment (FAB). This showed a molecular ion at m/z 470 (M^+ +1), together with a number of fragment ions. This data indicated that the product had the structure (329).



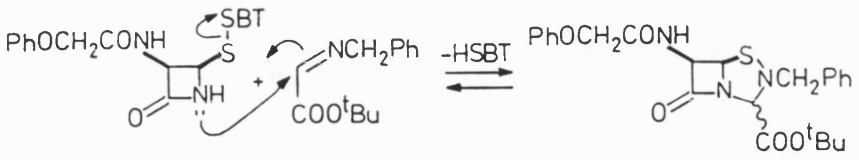
(329)



(330)

It is interesting that the expected intermediate (330) was not isolated - a possible explanation for this is that the

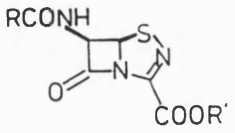
reaction may proceed in a concerted manner as shown in Scheme 82.



SCHEME 82.

2.4 Synthesis of 2-aza-1-thiapenems.

The final section of the research programme involved the study of approaches to the 2-aza-1-thiapenem system (331). It was appreciated at the outset that this bicyclic system would be highly strained and therefore highly reactive.



(331)

The proposed methodology involved nucleophilic attack by the azetidinone nitrogen of (305) at the cyano group of a cyanoformic ester (332). (Scheme 83). This methodology is similar to that used in the previous section in the preparation of t-butyl (2-benzyl-6β-phenoxyacetamido-(2-azapencillanate)).

(305)

(332) R'=CH_2Ph

(333) R'=^tBu

(331)

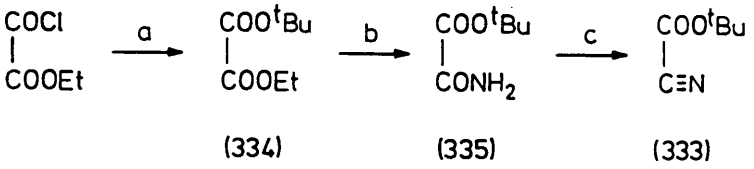
SCHEME 83.

The azetidinone (305) was available from work described previously. Benzyl cyanoformate (332) and t-butyl cyanoformate (333) were chosen as examples of cyanoformic esters for use in this study. Benzyl cyanoformate is commercially available, however t-butyl cyanoformate had to be prepared.

The chemistry of cyanoformates has not been extensively explored, and the reason for this seems to have been due to difficulty in their synthesis. However, in 1976 Childs & Weber published a general method for the synthesis of cyanoformates[166] in excellent yield via 18-crown-6 catalysed reaction of potassium cyanide with the corresponding chloroformate in dichloromethane solvent. Unfortunately the method fails in the case of t-butyl cyanoformate; this is attributed to the well-known instability of t-butyl chloroformate.

Therefore for the synthesis of t-butyl cyanoformate one is forced to use one of the older synthetic methods. Carpino reports a method in which t-butyl glyoxylate is used as an intermediate.[157] This involves conversion of t-butyl glyoxylate to t-butyl oximinoacetate using hydroxylamine hydrochloride, and subsequent dehydration using acetic

anhydride and triethylamine, giving t-butyl cyanoformate. However this method was not chosen, partly to conserve the limited stocks of t-butyl glyoxylate, which has to be made via a three stage procedure. The main reason for discounting this route was the potential hazard in the purification of t-butyl oximinoacetate. Carpino reports violent explosions during distillation on two separate occasions. Instead an alternative method of preparation of t-butyl cyanoformate was used, also due to Carpino.[167] (Scheme 84). This involves a three stage procedure, whereas the other (and more recent) Carpino procedure involves a five step synthesis from fumaric acid.



(334) (335) (333)

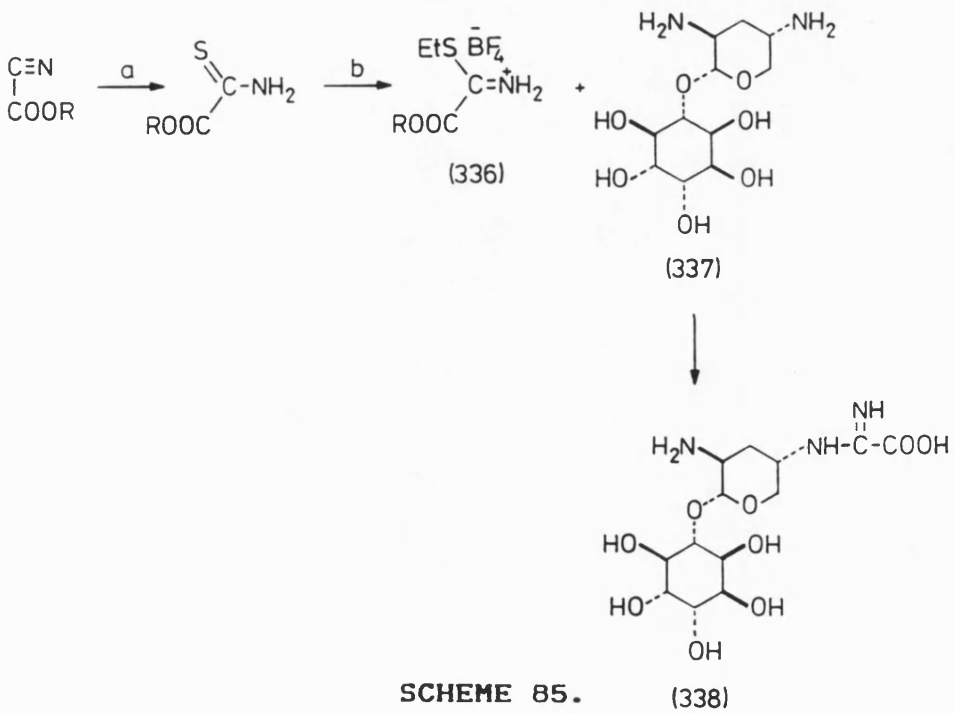
SCHEME 84.

a) ^{t}BuOH, py: b) NH_4OH: c) TFA, py.

Ethyl oxalyl chloride was added to a solution of t-butanol and pyridine in dichloromethane to give ethyl t-butyl oxalate (334). A solution of ethyl t-butyl oxalate in ethanol was treated with concentrated ammonium hydroxide to give t-butyl oxamate (335). This was treated with trifluoroacetic acid and pyridine, and the crude product was distilled to give t-butyl cyanoformate (333) in 33% overall yield.

A solution of the azetidinone (305) in tetrahydrofuran was treated with t-butyl cyanoformate under anhydrous conditions. The mixture was heated at reflux for several hours, but no reaction was observed and starting material was recovered. A similar procedure was carried out using benzyl cyanoformate, and again no reaction was observed. From these results it was deduced that the azetidinone nitrogen is not sufficiently nucleophilic for reaction to occur. Therefore a way of activating the cyanoformic ester towards nucleophilic attack was sought.

Thus attention was turned to Ohno's synthesis of kasugamycin,[168] in which benzyl cyanoformate was used as a starting material. The cyano group was selectively activated by conversion to the thiooxamimidate (336), and nucleophilic substitution by the amino group of kasuganobiosamine (337) to give kasugamycin (338). (Scheme 85).



SCHEME 85.

a) H_2S; or BzOH/HCl then P_2S_5: b) Et_3OBF_4.

It was decided that benzyl- and t-butyl thioxamimidates (336, R=Bz, t-Bu) should be prepared and the reaction of these with the azetidinone (305) studied.

An excess of hydrogen sulphide was condensed into a vessel containing a solution of the cyanoformate. The vessel was then sealed and left at room temperature for 24 hours. The crystalline product was then treated with triethyloxonium tetrafluoroborate according to Ohno's procedure.

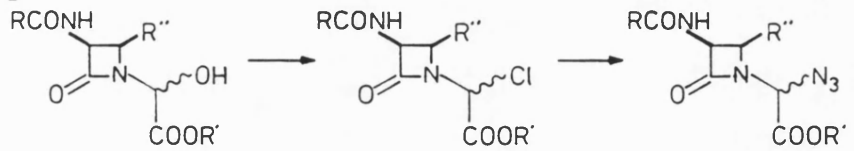
Triethyloxonium tetrafluoroborate is very hygroscopic, and although it is commercially available as a solution in dichloromethane it is preferable to prepare it[169] just prior to use. It is a useful reagent which readily ethylates ethers, sulphides, nitriles, ketones, esters and amides on oxygen, nitrogen or sulphur to give onium tetrafluoroborates (as in the above example) which can react with nucleophilic reagents to give useful products.[170] Another example of its utility is in the conversion of amides to amines under mild conditions - treatment of the amide with the reagent gives an imino ether tetrafluoroborate which is easily hydrolysed to the corresponding amine and ester.

Benzyl thiooxamimidate (336, R=Bz), was added to a solution of the azetidinone (305) in tetrahydrofuran under anhydrous conditions. The reaction mixture was heated at reflux for several hours, but no reaction was observed, and starting material was recovered. A similar procedure was carried out with t-butyl thiooxamimidate, again no reaction was observed.

Despite having activated the substrate it appears that the azetidinone nitrogen is still not nucleophilic enough for reaction to occur. Although Ohno has reported the reaction of thiooxamimidates with a variety of nucleophiles,[168,171] it must be noted that in most of these the nucleophile used

was a primary amine, except for one occasion where formylhydrazine was used. Although the ring strain in azetidinones reduces the extent of delocalisation of the lone electron pair on nitrogen into the amide bond, this obviously does not occur to sufficient extent to allow nucleophilic substitutions such as those attempted here.

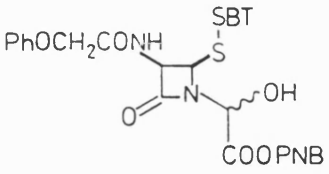
Although the above attempts towards the 2-aza-1-thiapenem system were unsuccessful, a surprising result was observed in the course of other work. The 1-(1-azetidin-2-one)-yl-1-hydroxyacetate (339, R"= SSBT) was prepared for use as an intermediate in 2-aza-1-thiapenam synthesis. There were no references to this compound in the literature, although similar compounds have been prepared. There are several examples[172] of the conversion of such compounds into the corresponding azides (341) via the chloride (340). (Scheme 86).



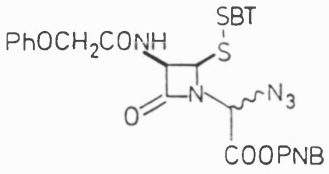
(339) (340) (341)

SCHEME 86.

As no references to the azide (342) were found in the literature, it was decided that the preparation of this compound should be attempted.



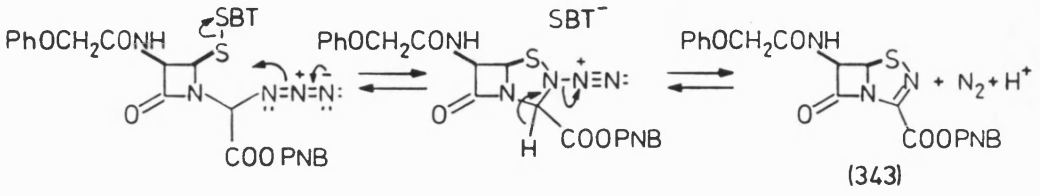
(320)



(342)

A solution of the hydroxyacetate (320) in tetrahydrofuran was treated with thionyl chloride and 2,6-lutidine under anhydrous conditions. The crude product was reacted immediately with sodium azide, and after rapid chromatography using silica gel deactivated with 10% water, a small amount of product was obtained. Surprisingly no azide N=N stretching frequency was observed in the infra red spectrum of this product. TLC data and the absence of the OH stretching frequency in the IR spectrum confirmed that the isolated compound was not the hydroxyacetate starting material. The nmr spectrum of the product showed the protons on the β-lactam ring at 5.52 and 5.93ppm; two quartets at 4.57 and 5.53ppm corresponding to the CH_2 protons on the phenoxyacetamido and p-nitrobenzyl groups; and a total of 9 aromatic protons. This indicates that the S-benzothiazolyl moiety has been eliminated.

On the basis of the above spectroscopic data the product was tentatively assigned the structure (343). A possible mechanism for the formation of this product is shown in Scheme 87.



(343)

SCHEME 87.

2.5 Summary.

Using the Hoechst methodology for the synthesis of the 7-unsubstituted 2-aza-1-thiacephems as a starting-point, synthetic methods for the preparation of 7β-acylamido-2-aza-1-thiacephems were explored. Several such compounds, protected as p-nitrobenzyl or methyl esters, were prepared. Attempts were made to deprotect these, although in only one case was the isolation of the free acid successful. This compound was found to have weak antibacterial activity against gram positive organisms.

It is not altogether surprising that attempts to deprotect these 2-aza-1-thiacephem esters led to only non-β-lactam containing products (with the one exception). It has already been noted that 2-aza-1-dethiacephems have limited stability as they are vinylogous carbamic acids. It was thought that preparation of 2-aza-1-thiacephems having a 2-acyl substituent would increase the stability of the bicyclic system, thereby facilitating the deprotection step. This was attempted, but it was found that the synthesis of the required intermediate failed.

The second part of the work involved new approaches to novel penam and penem derivatives. The synthesis of 2-aza-1-thia-penams involved two complementary strategies: firstly *in situ* conversion of (3-phenoxyacetamido-4-(2'-dithiobenzo-thiazolyl)azetidin-2-on-1-yl)-1-hydroxyacetates to the corresponding chloride, then treatment with an amine, was studied. The second approach involved reaction of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)azetidin-2-one with imines derived from glyoxylic esters. Both of these methods met with some measure of success, although the fundamental lack of stability of the highly-strained, reactive 2-aza-1-thiapenam system caused problems.

It was envisaged that a possible approach to the 2-aza-1-thiapenems involved reaction of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)azetidin-2-one with a cyanoformic ester. In practice it was found that the azetidinone nitrogen was not sufficiently nucleophilic to attack at the cyano group of the cyanoformate. An attempt was made to activate the said cyano group, but nucleophilic attack still did not take place. However, a small amount of compound thought to be a 2-aza-1-thiapenem was isolated during the attempted preparation of 3-(phenoxyacetamido-4-(2'-dithiobenzothiazolyl)azetidin-2-on-1-yl)-1-azido-acetate. The desired azide was not isolated. As expected, the isolated derivative was of limited stability.

3. EXPERIMENTAL.

Infra red spectra were taken on a Perkin Elmer 1310 grating spectrophotometer, using a 0.05mm polystyrene film as calibration.
Routine proton nuclear magnetic resonance spectra were taken on either a Hitachi-Perkin Elmer high resolution R24B spectrometer, or on a Varian Anaspec EM360 spectrometer. High resolution nuclear magnetic resonance spectra were taken on either a Jeol FX90Q or Jeol GMNGXFT 270 spectrometer.
Mass spectra were taken using a VG Analytical 7070E with VG 2000 data system. Spectra were routinely taken using Fast Atom Bombardment (FAB). Other ionisation techniques used were Electron Impact ionisation (EI) at an ionising potential of 70eV, or Chemical Ionisation (CI) using iso-butane as reactant gas.
Melting points are uncorrected.

Further analytical data and assignments of nmr and mass spectra are to be found in Appendix 3.

Preparation of penicillin G sulphoxide methyl ester (285).

Penicillin G potassium salt (50g) in DMF (290ml) was stirred with methyl iodide (19ml, 1.1 equiv.) for 4 hours. The solution was filtered through Celite, dissolved in ethyl acetate (400ml), and extracted with water (4x150ml). The organic layer was dried ($MgSO_4$) and evaporated in vacuo. The crude methyl ester was dissolved in chloroform (300ml) and stirred at 0°C. m-Chloroperbenzoic acid (12.0g, 1.1 equiv.) in chloroform (300ml) was added dropwise over 1 hr, and the reaction mixture was stirred for a further 30 min. The solution was washed with saturated sodium bicarbonate solution (2x100ml) and water (100ml). The crude product was evaporated in vacuo, and recrystallised from ethyl acetate, giving 17.12g (35%) penicillin G sulphoxide methyl ester as pale yellow crystals.

mp 118°C

ν_{max} (nujol) 3400, 1782, 1755, 1710, 1685cm^{-1}

δ ($CDCl_3$) 1.11 (s, 3H), 1.61 (s, 3H),3.52 (s, 2H), 3.72 (s, 3H), 4.59 s, 1H), 4.94 (d, 1H, J=6Hz), 5.93 (dd, 1H, J_1=10Hz, J_2=6Hz), 7.23 (s, 5H), 7.38 (d, 1H, J=10Hz).

m/z (EI) 364.02 (M^+).

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(methyl-3'-methylbut-2'-enoate)] azetidin-2-one (286).

Sulphoxide (285) (15.0g) was treated with 2-mercaptobenzothiazole (7.35g, 1 equiv.) in toluene (375ml) at reflux for 1.5 hr. The reaction mixture was cooled, evaporated in vacuo, and recrystallised from a small quantity of toluene, yielding 12.93g (61%) dithioazetidinone (286) as pale yellow crystals.

mp 133°C

ν_{max} (nujol) 3250, 1780, 1750, 1655cm^{-1}

δ ($CDCl_3$) 1.78 (s, 3H), 3.17 (s, 1H), 3.42 (s, 2H), 3.43 (s, 3H), 4.70 (d, 1H, J=16Hz), 4.84 (d, 1H, J=16Hz), 4.95 (dd, 1H, J_1=8Hz, J_2=3Hz), 5.11 (d, 1H, J=3Hz), 6.67-7.51 (m,

9H), 8.62 (d, 1H, J=8Hz).
m/z (EI) 515.81 (M^+).

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(methyl-3-oxobutanoate)]azetidin-2-one (287).

A solution of dithioazetidinone (286) (12.1g) in dichloromethane (350ml) was treated with ozonised oxygen at -78°C for 2.5hr. The ozonide was reduced using dimethyl sulphide. The reaction mixture was evaporated in vacuo, and purified by column chromatography on silica using 60% ethyl acetate/60-80 petrol as eluant, giving 8.62g of the enol (287) (71%) as a pale yellow foam.
ν_{max} ($CHCl_3$ soln.) 3300, 1780, 1665, 1615cm^{-1}
δ ($CDCl_3$) 2.22 (s, 3H), 3.35 (s, 3H), 3.42 (s, 2H), 4.74 (dd, 1H, J_1=8Hz, J_2=5Hz), 5.19 (d, 1H, J=5Hz), 7.1-7.6 (m, 10H), 10.8 (s, 1H).
m/z (+ve FAB) 516.81 (M^+).

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(methyl-3'-methanesulphonylbut-2'-enoate)]azetidin-2-one (288).

A solution of the enol (287) (5.49g) in dichloromethane (150ml) was treated with mesyl chloride (1.14ml, 1 equiv.) and triethylamine (1.78ml, 1 equiv.) under nitrogen at -15°C for 18 hr. The reaction mixture was filtered through Celite, evaporated in vacuo., and purified by chromatography on silica using 60% ethyl acetate/60-80 petrol as eluant, giving 2.86g (45%) of the mesylate (288, mixture of geometrical isomers), as a pale yellow foam.
ν_{max} ($CHCl_3$ soln) 3380, 1785, 1740, 1680 cm^{-1}
δ ($CDCl_3$) 1.22 (s, C=CMe, minor isomer), 2.04 (s, C=CMe, major isomer), 2.59 (s, OMs, minor isomer), 3.28 (s, OMs, major isomer), 3.59 (s, 2H), 3.66 (s, CO_2Me, major isomer), 3.81 (s, CO_2Me, minor isomer), 5.12 (dd, 1H, J_1=8Hz, J_2=4.5Hz), 5.61 (d, 1H, J=4.5Hz), 7.20-8.00 (m, 10H).

m/z (+ve FAB) 594.93 (M^+).

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(methyl-3'-phenylamidobut-2'-enoate)] azetidin-2-one (289).

A solution of mesylate (288) (1.0g) in dichloromethane (10ml) was stirred at 0°C under nitrogen. Phenylamine (0.16ml, 1 equiv.) and triethylamine (0.24ml, 1 equiv.) were added and stirring was continued for 1 hr. The reaction mixture was evaporated *in vacuo* and purified by chromatography on silica using 60% ethyl acetate/60-80 petrol as eluant, yielding 0.35g (35%) of the enamine (289, mixture of geometrical isomers) as a pale yellow foam.

ν_{max} ($CHCl_3$ soln.) 3290, 1772, 1674, 1602, 1580cm^{-1}

δ ($CDCl_3$) 1.23 (s, C=CMe, minor isomer), 2.41 (s, C=CMe, major isomer), 3.47 (s, 2H), 3.61 (s, CO_2Me, major isomer), 3.66 (s, CO_2Me, minor isomer), 4.59 (dd, 1H, J_1=7Hz, J_2=5Hz) 5.44 (d, 1H, J=5Hz), 6.92-7.32 (m, 9H), 8.83 (d, 1H, J=7Hz).

m/z (+ve FAB) 592.05 (M^+).

Preparation of methyl[2-phenyl-7β-phenylacetamido-2-aza cephalosporanate] (290).

The enamine (289) (0.3g) was stirred vigorously in benzene (distilled, 200ml) and finely divided silver acetate (0.3g) was added in one portion. The reaction mixture was heated under reflux for 1hr, then filtered through Celite and evaporated *in vacuo*. Chromatography on silica using 50% ethyl acetate/ 60-80 petrol as eluant gave 28mg (13%) of the 2-aza-1-thiacephem (290) as a cream coloured solid.

ν_{max} (nujol) 3340, 1787, 1720, 1690 cm^{-1}

δ ($CDCl_3$) 2.12 (s, 3H), 3.51 (s, 2H), 3.79 (s, 3H), 4.76 (d, 1H, J=5Hz), 5.89 (m, 2H), 6.82-7.37 (m, 10H).

m/z (high res. EI) 423.1245 (M^+).

Preparation of penicillin G sulphoxide p-nitrobenzyl ester.

A solution of penicillin G potassium salt (50.0g) and p-nitrobenzyl bromide (32g, 1.1 equiv.) in DMF was stirred overnight at room temp. The reaction mixture was dissolved in dichloromethane (250ml), filtered through Celite, washed with dil. HCl (2x50ml), satd. sodium bicarbonate soln. (2x50ml) then water (4x100ml) and dried ($MgSO_4$). The crude product was evaporated in vacuo, dissolved in chloroform (500ml) and stirred at 0°C. A solution of mCPBA (25.5g, 1.1 equiv.) in chloroform (500ml) was added dropwise, and the reaction mixture was stirred for a further 30 min. The solution was washed with potassium iodide soln. (50ml), sodium thiosulphate soln. (2x100ml), satd. sodium bicarbonate soln. (2x100ml) and water (2x100ml); dried ($MgSO_4$) and evaporated in vacuo to give 29.3g (45%) of penicillin G sulphoxide PNB ester as a pale yellow crystalline solid.

ν_{max} (nujol) 3360, 1785, 1750, 1690cm^{-1}

δ ($CDCl_3$) 1.10 (s, 3H), 1.64 (s, 3H), 3.52 (s, 2H), 4.65 (s, 1H), 4.93 (d, 1H, J=4Hz), 5.25 (s, 2H), 5.95 (dd, 1H, J_1=4Hz, J_2=10Hz), 7.1-8.2 (m, 10H).

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-methylbut-2'-enoate)] azetidin-2-one.

Penicillin G sulphoxide PNB ester (25.0g) was treated with 2-mercaptobenzothiazole (8.6g, 1 equiv.) in toluene (500ml) under reflux for 1.5 hr. The reaction mixture was cooled, evaporated in vacuo, and recrystallised from ethyl acetate, yielding 23.86g (73%) of the above dithioazetidinone as pale yellow crystals.

ν_{max} (nujol) 3270, 1790, 1755, 1660cm^{-1}

δ ($CDCl_3$) 1.95 (s, 3H), 3.71 (s, 2H), 5.04 (s, 2H), 5.14 (s, 1H), 5.18 (d, 1H, J=3Hz), 5.31 (s, 1H), 5.43 (dd, 1H, J_1=3Hz, J_2=11Hz), 7.2-8.3 (m, 14H).

m/z (70eV EI) 332 (no molecular ion observed). (no spectrum obtained with +ve or -ve FAB).

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3-oxobutanoate)] (292)

A solution of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-methylbut-2'-enoate)] azetidin-2-one (12.0g) in dichloromethane (350ml) was treated with ozonised oxygen at -78°C for 2.5hr. The ozonide was reduced using dimethyl sulphide. The reaction mixture was evaporated in vacuo, and purified by column chromatography on silica using 60% ethyl acetate/60-80 petrol as eluant, giving 8.55g (71%) of the enol (292) (71%) as a pale yellow foam.

ν_{max} ($CHCl_3$ soln.) 3430, 3310, 1760, 1735, 1670cm^{-1}

δ ($CDCl_3$) 2.30 (s, 3H), 3.52 (s, 2H), 5.05 (d, 1H, J=4Hz), 5.20 (s, 2H), 5.30 (dd, 1H, J_1=4Hz, J_2=9Hz), 6.8-7.9 (m, 13H), 11.8 (s, 1H)

m/z (70eV EI) 359 (corresponding to the fragment $[PhCH_2CONHCH{=}CHS\text{-}SBT]^+$). No spectrum obtained with +ve or -ve FAB.

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-phenylamidobut-2'-enoate)] azetidin-2-one.

A solution of the enol (292) (1.75g) in dichloromethane (150ml) was treated with mesyl chloride (0.21ml, 1 equiv.) and triethylamine (0.38ml, 1 equiv.) under nitrogen at -15°C for 18 hr. The reaction mixture was filtered through Celite, evaporated in vacuo., and used in the next step without further purification. A solution of the crude mesylate in dichloromethane (100ml) was stirred at 0°C under nitrogen. Phenylamine (0.25ml, 1 equiv.) and triethylamine (0.38ml, 1 equiv.) were added and stirring was continued for 1 hr. The reaction mixture was evaporated in vacuo and purified by chromatography on silica using 50% ethyl acetate/60-80 petrol as eluant, yielding 0.61g (32%) of the above enamine as a pale yellow foam. The product was

shown to be a mixture of geometrical isomers.
ν_{max} ($CHCl_3$ soln.) 3420, 3290, 1770, 1730, 1670cm^{-1}
δ (60 MHz, $CDCl_3$, major isomer) 2.38 (s, 3H), 3.51 (s, 2H), 4.78 (dd, 1H, J_1=9Hz, J_2=5Hz), 4.94 (s, 2H), 5.02 (bs, 1H), 5.38 (d, 1H, J=5Hz), 6.64-7.99 (m, 18H), 9.1 (bs, 1H).
The resonance at 5.02ppm was removed by deuterium exchange.
m/z (70eV EI) 544 (M^+-HSBT). No molecular ion with either +ve or -ve FAB.

Preparation of 4-nitrobenzyl[2-phenyl-7β-phenylacetamido-2-aza-1-thiacephalosporanate] (293).

3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-phenylamidobut-2'-enoate)] azetidin-2-one (358mg) was dissolved in dry benzene (1l) and stirred vigorously under nitrogen. Finely divided silver acetate (400mg) was added, the reaction mixture was heated under reflux for 30 min, filtered through Celite and evaporated *in vacuo*. The product was recrystallised from ethyl acetate to give 56mg of the 2-aza-1-thiacephem (289) (21%) as a cream coloured solid.
ν_{max} (nujol) 3270, 1785, 1710, 1660cm^{-1}
δ (60MHz, d^6DMSO) 2.09 (s, 3H), 3.44 (s, 2H), 4.89 (d, 1H, J=7Hz), 5.28 (s, 2H), 5.85 (dd, 1H, J_1=7Hz, J_2=9Hz), 6.86-8.17 (m, 14H), 8.82 (d, 1H, J=9Hz).
m/z 70eV E.I. - no molecular ion, but peaks at m/z 512, 410, 392; corresponding to loss of S, $PhCH_2CONH$, and $OCH_2C_6H_4NO_2$. Also m/z 352; arising from cleavage of the β-lactam ring. (no peaks were observed with either +ve or -ve FAB.)

Preparation of potassium [2-phenyl-7β-phenylacetamido-2-aza-1-thiacephalosporanate] (294).

Potassium hydrogen carbonate (28mg, 1 equiv.) and 10% palladium on carbon (302mg) were added to a suspension of the 2-aza-1-thiacephem (293) (150mg) in ethyl acetate (5ml) and

water (5ml). The mixture was treated with hydrogen at 50 psi in a Paar hydrogenator for 1.5 hr. The reaction mixture was filtered through Celite, the aqueous layer was separated, washed with ethyl acetate (2x5ml), and evaporated in vacuo to give 25mg of the 2-aza-1-thiacephem (294) (21%) as a white solid.

m.p. 187° C.

ν_{max} (nujol) 3280, 1785, 1710, 1660cm^{-1}

δ (D_2O, 250MHz) 1.63 (s, 3H), 4.81 (q, 2H), 5.33 (d, 1H, J=4.5Hz), 5.96 (d, 1H, J=4.5Hz), 7.0-7.4 (m, 10H).

m/z (-ve FAB) m/z 365 ($M^+ - CO_2$).

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'(4-nitrobenzyl-3'-methylamidobut-2'-enoate)] azetidin-2-one.

A solution of the enol (292) (1.75g) in dichloromethane (150ml) was treated with mesyl chloride (0.21ml, 1 equiv.) and triethylamine (0.38ml, 1 equiv.) under nitrogen at -15° C for 18 hr. The reaction mixture was filtered through Celite, evaporated in vacuo., and used in the next step without further purification. Methylamine (an excess) was added to a stirred solution of the crude mesylate in dichloromethane (100ml) at -78° C. The reaction mixture was stirred and allowed to warm slowly to room temp. over a period of 1 hr. The reaction mixture was evaporated in vacuo and purified by chromatography on silica using 30% ethyl acetate/60-80 petrol as eluant, yielding 0.30g (18%) of the above enamine as a yellow oil. The product was used immediately in the preparation of the 2-methyl azacephem (299).

ν_{max} ($CHCl_3$ soln.) 3430, 3290, 1770, 1660, 1600cm^{-1}

δ (60MHz, $CDCl_3$) major isomer 2.28 (s, 3H), 2.86 (s, 3H), 3.57 (s, 2H), 4.87 (dd, 1H, J_1=7Hz, J_2=10Hz), 5.09 (bs, 1H), 5.23 (d, 1H, J=7Hz), 6.98-8.14 (m, 13H), 8.94 (d, J=10Hz).

Preparation of 4-nitrobenzyl [2-methyl-7β-phenylacetamido-2-azacephalosporanate] (299).

3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(4-nitrobenzyl-3'-methylamidobut-2'-enoate)] azetidin-2-one (300mg) was dissolved in dry benzene (1.5l) and stirred vigorously under nitrogen. Finely divided silver acetate (300mg) was added and the reaction mixture was heated under reflux for 50 min, then filtered through Celite and evaporated in vacuo. The crude product was purified by flash column chromatography on silica using 30% ethyl acetate/ 60-80 petrol as eluant, yielding 35mg (16%) of the cyclised product (299) as a white solid.

ν_{max} (nujol) 3240, 1730, 1705, 1635cm^{-1}

δ (60MHz, d^6 DMSO) 2.30 (s, 3H), 3.26 (s, 3H), 3.43 (s, 2H), 4.91 (d, 1H, J=6Hz), 5.18 (s, 2H), 5.81 (dd, 1H, J_1= 11Hz, J_2=6Hz), 6.97-8.16 (m, 9H), 8.78 (d, 1H, J=11Hz).

Preparation of penicillin V sulphoxide p-nitrobenzyl ester.

A solution of penicillin V potassium salt (57.2g) and p-nitrobenzyl bromide (35g, 1.1 equiv.) in DMF was stirred overnight at room temp. The reaction mixture was dissolved in dichloromethane (250ml), filtered through Celite, washed with dil. HCl (2x50ml), satd. sodium bicarbonate soln. (2x50ml) then water (4x100ml) and dried ($MgSO_4$). The crude product was evaporated in vacuo, dissolved in chloroform (500ml) and stirred at 0°C. A solution of mCPBA (34.6g, 1.1 equiv.) in chloroform (500ml) was added dropwise, and the reaction mixture was stirred for a further 30 min. The solution was washed with potassium iodide soln. (50ml), sodium thiosulphate soln. (2x100ml), satd. sodium bicarbonate soln. (2x100ml) and water (2x100ml); dried ($MgSO_4$) and evaporated in vacuo to give 65.7g (89%) of penicillin V sulphoxide PNB ester as a pale yellow crystalline solid.

ν_{max} (nujol) 3410, 1785, 1735, 1690cm^{-1}

δ ($CDCl_3$) 1.16 (s, 3H), 1.72 (s, 3H), 4.54 (s, 2H), 4.75 (s, 1H), 5.05 (d, 1H, J=5Hz), 5.37 (q, 2H), 6.13 (dd, 1H,

J_1=5Hz, J_2=10Hz), 6.9-8.3 (m, 10H).

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-methyl-but-2'-enoate)]azetidin-2-one (308).

Penicillin V sulphoxide PNB ester (20.0g) was treated with 2-mercaptobenzothiazole (6.67g, 1 equiv.) in toluene (500ml) under reflux for 1.5 hr. The reaction mixture was cooled, evaporated in vacuo, and recrystallised from ethyl acetate, yielding 18.42g (71%) dithioazetidinone (308) as pale yellow crystals.

mp 139-142°C

ν_{max} ($CHCl_3$ soln.) 1770, 1750, 1685cm^{-1}

δ (d^6 DMSO) 1.98 (s, 3H), 4.57 (q, 2H), 5.02 (d, 2H, J=3Hz), 5.22 (q, 2H), 5.24 (s, 1H), 5.47 (dd, 1H, J_1=5Hz, J_2=9Hz), 5.56 (d, 1H, J=5Hz), 6.9-8.2 (m, 14H).

m/z (+ve FAB) 484 (M^+-SBT).

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(4-nitrobenzyl)-3'-oxobutanoate))]-azetidin-2-one (295).

A solution of disulphide (308) (6.79g) in dichloromethane (150ml) was treated with ozonised oxygen at -78°C for 2.5 hrs. The ozonide was reduced with excess dimethyl sulphide. The reaction mixture was warmed slowly to room temp. and evaporated in vacuo. The crude product was purified by flash column chromatography using 40% ethyl acetate/ 60-80 petrol as eluant, giving 4.16g of the enol (295) (61%) as a colourless foam.

ν_{max} ($CHCl_3$ soln.) 3420, 2990, 1780, 1730, 1690cm^{-1}

δ (250MHz, $CDCl_3$) 2.38 (s, 3H), 4.62 (dd, 2H), 5.17 (dd, 2H), 5.19 (dd, 1H, J_1=5.2Hz, J_2=10.4Hz), 5.41 (d, 1H, J=5.0Hz), 6.93-8.05 (m, 13H), 7.40 (dd, 1H, J_1=10.6Hz, J_2=7.5Hz), 12.21 (s, 1H).

m/z (+ve FAB) 485 (M^+-HSBT).

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-methanesulphonylbut-2'-enoate))]azetidin-2-one.

A solution of enol (295) (1.2g) in dichloromethane (150ml) was stirred under nitrogen at -78°C. Mesyl chloride (0.16ml, 1.1 equiv.) then triethylamine (0.29ml, 1.1 equiv.) were added dropwise. The reaction mixture was stirred for 18 hr; during this time the reaction was allowed to warm slowly to room temp. The crude product was evaporated in vacuo. and purified by flash column chromatography using 40% ethyl acetate/60-80 petrol as eluant, giving 445mg of a pale yellow foam. The product was found to be a mixture of the desired mesylate and starting material.

ν_{max} ($CHCl_3$ soln.) 3430, 1780, 1735, 1690cm^{-1}

δ (250MHz, $CDCl_3$) major isomer: 2.59 (s, 3H), 3.19 (s, 3H), 4.63 (q, 2H), 5.06 (q, 2H), 5.10 (dd, 1H, J_1=5Hz, J_2=8Hz), 5.61 (d, 1H, J=5Hz), 6.2-8.2 (m, 14H).

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-(4-toluenesulphonyl) but-2'-enoate))]azetidin-2-one (297).

A solution of enol (295) (0.9g) in dichloromethane (150ml) was stirred under nitrogen at -78°C. Tosyl chloride (0.30g) then triethylamine (0.22ml, 1.1 equiv.) were added dropwise. The reaction mixture was stirred for 18 hr, during this time the reaction was allowed to warm slowly to room temp. The crude product was evaporated in vacuo. and purified by flash column chromatography using 40% ethyl acetate/60-80 petrol as eluant, giving 330mg (29%) of the tosylate (297) as a pale yellow foam. The product was found to be a mixture of geometrical isomers.

ν_{max} ($CHCl_3$ soln) 3410, 1780, 1725, 1695cm^{-1}

δ (250MHz, $CDCl_3$) major isomer: 1.6 (s, 3H), 2.49 (s, 3H), 4.54 (q, 2H), 5.25 (q, 2H), 5.58 (dd, 1H, J_1=5Hz, J_2=9Hz), 5.70 (d, 1H, J=5Hz), 6.8-8.3 (m, 18H).

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-trifluoromethanesulphonylbut-2'-enoate)]azetidin-2-one (298).

A solution of enol (294) (130mg) in dichloromethane (5ml) was stirred under nitrogen at -78°C. Trifluoromethanesulphonic anhydride (0.035ml, 1 equiv.) then N,N-diisopropylethylamine (0.040ml, 1 equiv.) were added dropwise. The reaction mixture was stirred for 15 min at -78°C, then warmed slowly to room temp. and evaporated in vacuo. The crude product was purified by flash column chromatography using 50% ethyl acetate/60-80 petrol as eluant, giving 103mg (69%) of the triflate (298) as a pale yellow foam. The product was found to be a mixture of geometrical isomers.

ν_{max} ($CHCl_3$ soln.) 3410, 2920, 1780, 1715, 1675 cm^{-1}

δ ($CDCl_3$) major isomer: 2.55 (s, 3H), 4.64 (dd, 2H), 5.02 (dd, 2H), 5.10 (dd, 1H, J_1=5.2Hz, J_2=2.2Hz), 5.73 (d, 1H, J=5.5Hz), 6.89-8.18 (m, 13H), 7.42 (dd, 1H, J_1=2.1Hz, J_2=7.0Hz).

m/z no molecular ion with EI or CI: no peaks in either +ve or -ve FAB.

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(4-nitrobenzyl)-3'-phenylamidobut-2'-enoate)]azetidinone (296, R=Ph).

A solution of the triflate (298) (0.70g) in dichloromethane (150ml) was stirred at -78°C under nitrogen. N,N-Diisopropylethylamine (0.18ml, 1.1 equiv.) and aniline (0.9ml, 1.1 equiv.) were added dropwise. The reaction mixture was stirred at -78°C for 30 min, warmed slowly to room temperature, then evaporated in vacuo. The crude product was purified by flash column chromatography using 40% ethyl acetate/60-80 petrol as eluant, to give 714mg (63%) of the enamine (296, R=Ph) as a yellow foam. The product was found to be a mixture of geometrical isomers.

ν_{max} ($CHCl_3$ soln.) 3430, 1775, 1725, 1685cm^{-1}

δ ($CDCl_3$) 2.45 (s, 3H), 4.63 (q, 2H), 4.85 (dd, 1H,

J_1=5Hz, J_2=8Hz), 5.21 (q, 2H), 5.53 (d, 1H, J=5Hz), 6.9-8.1 (m, 19H), 8.98 (s, 1H).

m/z no molecular ion with EI or CI: no peaks in either +ve or -ve FAB.

Preparation of 4-nitrobenzyl[2-phenyl-7β-phenoxyacetamido-2-azacephalosporanate] (300).

A solution of the enamine (296, R=Ph) (200mg) in benzene (11) was stirred vigorously under nitrogen. Silver acetate (250mg) was added in one portion and the reaction mixture was heated under reflux for 30 min. The reaction mixture was then cooled, filtered through Celite and evaporated in vacuo. The crude product was purified by flash column chromatography using 40% ethyl acetate/60-80 petrol as eluant, giving 33mg (22%) of the 2-aza-1-thiacephem (300) as a white solid.

ν_{max} ($CHCl_3$ soln.) 3280, 1795, 1725, 1680cm^{-1}

δ (270MHz, d^6 DMSO) 2.11 (s, 3H), 4.58 (s, 2H), 5.05 (d, 1H, J=5Hz), 5.41 (s, 2H), 6.06 (dd, 1H, J_1=5Hz, J_2=8Hz), 6.8-7.4 (m, 10H), 7.75 (d, 2H, J=9Hz), 8.25 (d, 2H, J=9Hz), 9.11 (d, 1H, J=8Hz).

m/z (70 eV E.I.) - no molecular ion, but peaks at m/z 528, 410, 408 ; corresponding to loss of S, $PhOCH_2CONH$, and $OCH_2C_6H_4NO_2$. Also m/z 352; arising from cleavage of the β-lactam ring. No peaks were observed in either +ve or -ve FAB.

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(4-nitrobenzyl)-3'-cyanomethylamidobut-2'-enoate)]azetidinone. (296, R=CH_2CN)

N,N-Diisopropylethylamine (0.048ml, 2 equiv.) and aminoacetonitrile hydrochloride (13mg, 1 equiv.) were stirred in dichloromethane (2ml) at room temperature for 30 min. The resulting solution was added with stirring to a solution of the triflate (298) (103mg) in dichloromethane (5ml) at -78°C under nitrogen. The reaction mixture was stirred at -78°C for 30 min, warmed slowly to room temperature, then

evaporated in vacuo. The crude product was purified by flash column chromatography using 50% ethyl acetate/60-80 petrol as eluant, to give 74mg (78%) of the above enamine as a yellow foam. The product was found to be a mixture of geometrical isomers.

ν_{max} ($CHCl_3$ soln.) 3420, 3300, 1775, 1700, 1680 cm^{-1}

δ ($CDCl_3$) major isomer: 2.60 (s, 3H), 4.21 (dd, 2H), 4.60 (dd, 2H), 4.81 (dd, 1H, J_1=4.8Hz, J_2=2.9Hz), 5.05 (dd, 2H), 5.46 (d, 1H, J=4.8 Hz), 6.94-8.04 (m, 13H), 8.22 (dd, 1H, J_1=5.5Hz, J_2=2.9Hz), 8.74 (t, 1H). The resonance at 8.74 ppm was removed by deuterium exchange.

m/z (+ve FAB) 613, 569, 555, 554 (corresponding to loss of Ph, $C_6H_5NO_2$, $PhOCH_2CO$ and $CH_2C_6H_4NO_2$ from M^+)

Preparation of 4-nitrobenzyl[2-cyanomethyl-7β-phenoxyacetamido-2-azacephalosporanate] (301).

A solution of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(4-nitrobenzyl)-3'-cyanomethylamidobut-2'-enoate)]azetidinone (296, R=CH_2CN) (510mg) in benzene (1500ml) was stirred vigorously under nitrogen. Silver acetate (510mg) was added in one portion and the mixture heated under reflux for 30 min. The reaction mixture was then cooled, filtered through Celite and evaporated in vacuo. The crude product was purified by flash column chromatography using 50% ethyl acetate/60-80 petrol as eluant, giving 119mg (29%) of the 2-aza-1-thiacephem (301) as a white solid.

ν_{max} ($CHCl_3$ soln.) 3400, 2900, 1790, 1720, 1700 cm^{-1}

δ (400MHz, $CDCl_3$) 2.41 (s, 3H), 4.23 (dd, 2H), 4.54 (s, 2H), 5.16 (d, 1H, J=4.6Hz), 5.34 (dd, 2H), 6.13 (dd, 1H, J_1=4.6Hz, J_2=3.6Hz), 6.88-8.26 (m, 9H), 7.04 (dd, 1H, J_1=3.6Hz, J_2=7.4Hz).

m/z (+ve FAB) m/z 388, 387, 371 (loss of $PhOCH_2CO$, $CH_2C_6H_4NO_2$, and $OCH_2C_6H_4NO_2$ from M^+).

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-methylbut-3'-enoate)] azetidinone (309).

A solution of the disulphide (308) (10.0g) in dichloromethane (150ml) was stirred at room temp. under nitrogen. Triethylamine (2.2ml, 1.1 equiv.) was added dropwise and the reaction mixture was stirred at room temp. for 45 min. An IR spectrum of the crude reaction mixture showed that the conversion was complete. The solution was washed with 5% aq. citric acid (2x100ml) then water (2x50ml), dried ($MgSO_4$) and evaporated *in vacuo*. The product was purified by flash column chromatography using 40% ethyl acetate/petrol as eluant, giving 6.23g (309) (62%) as pale yellow crystals.

mp 113°C (lit. 115°C).

ν_{max} ($CHCl_3$ soln.) 3420, 1770, 1725, 1690cm^{-1}

δ ($CDCl_3$ soln.) 2.18 (s, 3H), 2.23 (s, 3H), 4.58 (q, 2H), 5.08 (q, 2H), 5.12 (dd, 1H, J_1=8Hz, J_2=5Hz), 5.50 (d, 1H, J=5Hz), 6.8-8.0 (m, 14H).

m/z (+ve FAB) 551 ($M^+ - PhOCH_2$).

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-2'-oxoethanoate)]-azetidinone (310).

A solution of the disulphide (309) (6.23g) in methyl acetate was stirred at -78°C and ozonised oxygen was passed through the solution for 1.5hr. Excess ozone was removed from the solution with a stream of nitrogen. The reaction mixture was allowed to warm to room temp; washed with 10% aq. sodium bisulphite (100ml) then brine (2x100ml), dried ($MgSO_4$) and evaporated *in vacuo*. Recrystallisation from a small quantity of methyl acetate, gave 3.29g product (55%).

ν_{max} ($CHCl_3$ soln.) 1825, 1765, 1710cm^{-1}

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl) azetidinone (305).

The ozonisation product (310) (3.29g) was heated at reflux in a mixture of methanol (375ml), methyl acetate (50ml), and water (10ml) for 30 min. The reaction mixture was evaporated in vacuo. and purified by chromatography on silica gel. 20% Ethyl acetate/petrol eluted methyl-p-nitrobenzyl oxalate: the desired azetidinone (305) (1.12g, 51%) was obtained by eluting with ethyl acetate.

mp. 152-154°C (lit: 156-158°C)

ν_{max} (nujol) 3380, 1785, 1685cm^{-1}

δ (d^{8}THF) 4.60 (q, 2H), 5.31 (d, 1H, J=5Hz), 5.52 (dd, 1H, J_1=9Hz, J_2=5Hz), 6.9-7.9 (m, 9H), 8.23 (s, 1H), 8.48 (d, 1H, J=9Hz).

m/z (+ve FAB) 418 (M^++1).

Preparation of di-t-butyl fumarate (314).

2-Methoxyethyl ether (80ml), fumaric acid (10g) and conc. sulphuric acid (10ml) were placed in a pressure bottle, which was cooled in an acetone/CO_2 bath. Isobutylene (100ml) was condensed into the pressure bottle, which was sealed and shaken at room temp. for 48 hr. The vessel was then cooled, opened, and the contents poured into 2N sodium hydroxide (250ml). The aqueous soln. was extracted with ether (2x100ml), the ether extracts were combined, washed with water (7x100ml), dried ($MgSO_4$), evaporated in vacuo., and recrystallised from ether, giving 10.65g di-t-butyl fumarate (64%) as a white crystalline solid.

mp. 65-67°C (lit. 69-70°C).

ν_{max} (nujol) 1690cm^{-1}

δ ($CDCl_3$, 60MHz) 1.45 (s, 9H), 6.60 (s, 1H).

m/z (CI) 229 (M^++H), 173 (M^+-(t-Bu)).

Preparation of di-t-butyl tartrate (315).

A solution of di-t-butyl fumarate (314) (10.6g) in t-butanol (300ml) was stirred at room temp. and an aqueous soln. of potassium permanganate (5.08g in 300ml) was added dropwise. The reaction mixture was extracted with ether (2x600ml), the combined ether extracts were washed with water (3x250ml), dried ($MgSO_4$), evaporated in vacuo and recrystallised from petroleum ether, giving 5.85g di-t-butyl tartrate (48%) as a white crystalline solid.

mp. 82-83°C (lit. 84-85°C).

ν_{max} (nujol) 3500, 1730cm^{-1}

δ ($CDCl_3$, 60MHz) 1.50 (s, 9H), 3.15 (d, 2H), 4.40 (d, 2H).

m/z (CI) 263 (M^++H).

Preparation of t-butyl glyoxylate monohydrate (317).

Lead tetraacetate (9.76g) was added in one portion to a stirred solution of di-t-butyl tartrate (5.01g) in dry benzene (70ml). The reaction mixture was stirred for 3 hours at room temp. under a nitrogen atmosphere. Petroleum ether (150ml) was added, the mixture was stirred for 10 min then filtered. The filtrate was evaporated in vacuo. The crude product was distilled under reduced pressure, the fraction distilling at 49°C (12mmHg) was collected, giving 4.97g t-butyl glyoxylate monohydrate (317) (53%) as a viscous colourless oil.

ν_{max} ($CHCl_3$ soln.) 3440, 1745 cm^{-1}

δ ($CDCl_3$ soln.) 1.15 (s, 9H), 3.64 (m, 1H), 3.80 (m,1H), 5.16 (q, 1H).

m/z (CI) 149 (M^++H) at m/z 149.

Preparation of di-(4-nitrobenzyl) tartrate (318).

A mixture of tartaric acid (3.83g), 4-nitrobenzyl bromide (11.03g, 2 equiv.) and triethylamine (7.09ml, 2 equiv.) in DMF (120ml) was stirred at room temp. for 18hr. The reaction mixture was poured into water (600ml) and stirred for 1hr. The precipitate was filtered, washed with water and dried in

a vacuum oven, giving 10.1g di-(4-nitrobenzyl) tartrate (95%) as a white solid.

ν_{max} (nujol) 3400, 1700cm^{-1}

δ (60MHz, d^6 DMSO) 5.30 (s, 2H), 5.85 (bs, 2H), 7.60 (d, 2H), 8.10 (d,2H).

Preparation of 4-nitrobenzyl glyoxylate monohydrate (319).

Periodic acid (9.3g) was added to a stirred suspension of di-(4-nitrobenzyl) tartrate (10g) in THF (250ml) at 0°C. The reaction mixture was stirred for 4 hr., filtered through Celite and evaporated in vacuo. The crude product was purified by flash colunm chromatography on silica using 50% ethyl acetate/petrol as eluant, giving 3.94g 4-nitrobenzyl glyoxylate monohydrate (47%) as a white solid.

ν_{max} (nujol) 3460, 1705cm^{-1}

δ (60MHz, d^6 DMSO) 3.35 (s,1H), 4.65 (d, 1H), 5.78 (d, 1H), 7.60 (d, 2H), 8.16 (d, 2H).

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzo-thiazolyl)-1-[2'-(p-nitrobenzyl)-2'-hydroxyacetate)] azetidin-2-one (320).

A solution of the azetidinone (305) (0.68g) in anhydrous tetrahydrofuran (100ml) was stirred under a nitrogen atmosphere and in the presence of molecular sieves. A solution of p-nitrobenzyl glyoxylate monohydrate (319) (0.66g; an excess) in anhydrous tetrahydrofuran (10ml) was added dropwise. The reaction mixture was stirred at room temperature for 18 hr, filtered and evaporated in vacuo. The crude product was purified by flash column chromatography using 40% ethyl acetate/petrol as eluant, giving 0.7g (69%) product as a colourless foam.

ν_{max} ($CHCl_3$ soln.) 3530, 3390, 1775, 1745 1680cm^{-1}

δ ($CDCl_3$) 4.71 (m, 2H), 5.32 (m, 2H), 5.38 (dd, 1H, J_1=5Hz, J_2=8Hz), 5.48 (d, 1H, J=5Hz), 5.70 (s, 1H), 6.86 (bs, 1H), 7.0-8.3 (m, 13H), 9.28 (dd, 1H, J_1=8Hz, J_2=8Hz).

m/z (+ve FAB) 627 ($M^+ +1$).

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(t-butyl)-2'-hydroxyacetate)] azetidin-2-one (321).

A solution of the azetidinone (305) (2.0g) in anhydrous tetrahydrofuran (200ml) was stirred under a nitrogen atmosphere and in the presence of molecular sieves. A solution of t-butyl glyoxylate monohydrate (317) (1.0g; an excess) in anhydrous tetrahydrofuran (10ml) was added dropwise. The reaction mixture was stirred at room temperature for 18 hr, filtered and evaporated in vacuo. The crude product was purified by flash column chromatography using 50% ethyl acetate/petrol as eluant, giving 1.28g (49%) product as a colourless foam.

ν_{max} ($CHCl_3$ soln.) 3500, 3400, 1775, 1725, 1685cm^{-1}

δ ($CDCl_3$) 1.30 and 1.49 (two singlets, due to ^tBu group of each enantiomer, total 9H), 4.57 (m, 2H), 5.28 (s, 1H), 5.50 (m, 2H), 5.60 (d, 1H, J=8Hz), 5.64 (dd, 1H, J_1=5Hz, J_2=8Hz), 6.9-8.0 (m, 10H), 9.28 (s, 1H).

m/z (+ve FAB) 548 ($M^+ +1$).

Preparation of p-nitrobenzyl(2-benzyl-6β-phenoxyacetamido-2-aza-1-thiapenicillanate) (322).

A solution of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(p-nitrobenzyl)-2'-hydroxyacetate)] azetidin-2-one (321, 0.65g) in dry tetrahydrofuran was stirred under a nitrogen atmosphere at -15°C. To this were added 2,6-lutidene (0.18ml, 1.5 equiv.) and a solution of thionyl chloride (0.11ml, 1.5 equiv.) in dry tetrahydrofuran (3ml). The reaction mixture was warmed to room temperature over a period of 2hr., filtered and evaporated in vacuo. The material was dissolved in dichloromethane (10ml) and stirred under a nitrogen atmosphere at -15°C. Benzylamine (0.11ml, 1 equiv.) was added and the reaction mixture was stirred at room temperature for 18hr, then evaporated in

vacuo. Flash column chromatography yielded two β-lactam containing products. The less polar of these was isolated in 9% yield (51.6mg) and was thought to be p-nitrobenzyl(2-benzyl-6β-phenoxyacetamido-2-aza-1-thiapenicillanate) (322). In addition, a product thought to be 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((p-nitrobenzyl)-2-aminobenzylacetate)] azetidin-2-one (323) was isolated in 10% yield (71mg).

(322)

ν_{max} ($CHCl_3$ soln.) 3500, 3480, 1760, 1740, 1680cm^{-1}

δ ($CDCl_3$) 4.52 (q, 2H), 5.21 (q, 2H), 5.45 (q, 2H), 5.61 (dd, 1H, J_1=5Hz, J_2=8Hz), 5.82 (d, 1H, J=5Hz), 6.65 (d, 1H), 6.8-8.2 (m, 15H).

m/z (+ve FAB) 549 (M^++1).

(323)

ν_{max} ($CHCl_3$ soln.) 3500, 3480, 1760, 1740, 1680cm^{-1}

δ ($CDCl_3$) 4.04 (m, 2H), 4.56 (m, 2H), 4.83 (s, 1H), 5.13 (m, 2H), 5.24 (dd, 1H, J_1=5Hz, J_2=7Hz), 5.32 (d, 1H, J=5Hz), 6.9-8.0 (m, 18H), 8.06 (d, 1H), 8.20 (m, 1H).

m/z (+ve FAB) 625 (M^+-Bz), 609 (M^+-$BzNH_2$), 548 (M^+-HSBT).

Preparation of t-butyl(1-benzyliminoacetate) (306, R'=Bz, R''=^{t}Bu.

A mixture of benzylamine (0.51g, 1.1 equiv.), dichloromethane (10ml) and activated molecular sieves was stirred at 0°C under a nitrogen atmosphere. t-Butyl glyoxylate (317) (0.60g, 1 equiv.) in dichloromethane (10ml) was added with stirring over a period of 20 min. The mixture was stirred at room temperature for a further 90 min, then filtered and evaporated in vacuo. Purification by flash column chromatography on silica gel deactivated with 10% water gave

the imine (306, R'=Bz, R"=^{t}Bu) (0.67g, 66%) as a pale yellow oil.

ν_{max} (film) 1710, 1640cm^{-1}

δ ($CDCl_3$ soln.) 1.54 (s, 9H), 4.83 (s, 2H), 7.20-7.30 (m, 5H), 7.63 (s, 1H).

m/z (CI) 220 (M^++1).

Preparation of t-butyl(2-benzyl-6β-phenoxyacetamido-2-aza-1-thiapenicillanate) (325).

t-Butyl(1-benzyliminoacetate) (306, R'=Bz, R"=^{t}Bu) (129mg) in dry tetrahydrofuran (5ml) was added to a stirred solution of the azetidinone (305) (252mg, 1 equiv.) under a nitrogen atmosphere. The reaction mixture was heated under reflux for 5 hr., then evaporated in vacuo. and purified by flash column chromatography on silica, using 50% ethyl acetate/petrol as eluant. 32mg (12%) product was obtained as a white gum.

ν_{max} ($CHCl_3$ soln.) 3390, 1765, 1720, 1690cm^{-1}

δ (d^8 THF, major isomer) 1.47 (s, 9H), 4.1 (q, 2H), 4.63 (q, 2H), 4.92 (s, 1H), 5.15 (dd, 1H, J_1=5Hz, J_2=8Hz), 5.23 (d, 1H, J=5Hz), 6.9-7.9 (m, 10H), 8.56 (d, 1H, J=8Hz).

m/z (+ve FAB) 470 (M^++1).

Preparation of ethyl t-butyl oxalate (334).

t-Butanol (6.9ml) and pyridine (5.9ml) were stirred in dichloromethane (15ml) at 0°C. Ethyl oxalyl chloride (8.2ml) was added dropwise over a period of 1 hr. The reaction mixture was stirred at 0°C for 4 hr., then washed with water (3x50ml), dried ($MgSO_4$) and evaporated in vacuo. The crude product was distilled under reduced pressure, the fraction distilling at 58-60°C (2.5mmHg) was collected, giving 9.26g ethyl t-butyl oxalate (73%) as a colourless oil.

ν_{max} ($CHCl_3$ soln.) 1755, 1735cm^{-1}

m/z (iso-but. CI) 175 (M^++1).

Preparation of t-butyl oxamate (335).

A solution of ethyl t-butyl oxalate (334) (9.0g) in ethanol (5ml) was stirred at room temp. Conc. ammonium hydroxide (4ml) was added dropwise, and stirring was continued for 18 hr. The reaction mixture was extracted with dichloromethane (3x50ml), the extracts were combined, washed with water (3x25ml), dried ($MgSO_4$) and evaporated *in vacuo*., giving 9.26g t-butyl oxamate (85%) as a white solid.

ν_{max} (nujol) 1745, 1690cm^{-1}

Preparation of t-butyl cyanoformate (333).

A mixture of t-butyl oxamate (335) (8.8g) and pyridine (11ml) was stirred at 0°C. Trifluoroacetic anhydride (10.3ml) was added dropwise and the reaction was stirred for 15 min at 0°C, then a further 15 min at room temp. Diethyl ether (40ml) was added, the solution was stirred for 5 min then washed with sodium bicarbonate solution (2x15ml), then with water (2x15ml), dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was distilled under reduced pressure, the fraction distilling at 30°C (35mmHg) was collected, giving 4.0g t-butyl cyanoformate (52%) as a colourless oil.

ν_{max} (film) 2220, 1760cm^{-1}

analysis found C 57.0%, H 7.2%, N 11.3%

calc. C 56.7%, H 7.1%, N 11.0%

Preparation of benzyl thioxamidate.

A solution of benzyl cyanoformate (1ml) in dry tetrahydrofuran (10ml) was placed in a thick-walled glass tube, which was cooled in an acetone/CO_2 bath. Hydrogen sulphide was bubbled into the solution for 10 min., then the tube was sealed and allowed to stand at room temperature for 18hr. The tube was cooled, opened, and excess hydrogen sulphide was removed by passing dry nitrogen through the reaction mixture. Evaporation *in vacuo* and recrystallisation from ethyl acetate gave 1.14g benzyl thioxamidate (64%) as a bright

yellow solid.

mp. 86° C

ν_{max} (nujol) 3410, 3300, 1720cm^{-1}

δ (60MHz, $CDCl_3$) 5.25 (s,2H), 7.35 (s, 5H), 8.20 (bs, 2H).

^{13}C nmr ($CDCl_3$) 187, 159, 134.3, 128.7, 128.4, 69.3.

m/z (EI) 195 (M^+), 167.

Preparation of benzyl thiooxamimidate (336, R=Bz).

A mixture of benzyl thioxamidate (0.5g) and triethyloxonium tetrafluoroborate (0.45g, 1 equiv.) in dry dichloromethane (20ml) was stirred for 2hr at room temperature under a nitrogen atmosphere. The solvent was removed in vacuo. and the crude product recrystallised from dichloromethane/hexane to give 0.61g (76%) of the product as a white solid.

mp. 78-82° C, dec. (lit. 75-83° C).

ν_{max} (nujol) 3290, 3160, 1775, 1620cm^{-1}

δ (60 MHz, $CDCl_3$) 1.4 (t, 3H), 3.35 (q, 2H), 5.4 (s, 2H), 7.3 (s, 5H), 9.55 (bs, 2H).

Preparation of t-butyl thioxamidate.

A solution of t-butyl cyanoformate (2.2g) in dry tetrahydrofuran (5ml) was placed in a thick-walled glass tube, which was cooled in an acetone/CO_2 bath. Hydrogen sulphide was bubbled into the solution for 10 min., then the tube was sealed and allowed to stand at room temperature for 18hr. The tube was cooled, opened, and excess hydrogen sulphide was removed by passing dry nitrogen through the reaction mixture. Evaporation in vacuo and recrystallisation from ethyl acetate gave 2.34g t-butyl thioxamidate (84%) as a bright yellow solid.

ν_{max} (nujol) 3410, 3300, 1720cm^{-1}

δ (60MHz, $CDCl_3$) 1.55 (s, 9H), 8.1 (bs, 2H).

Preparation of t-butyl thioxamimidate (336, R=^{t}Bu).

A mixture of t-butyl thioxamidate (0.25g) and triethyloxonium tetrafluoroborate (0.3g, 1 equiv.) in dry dichloromethane (10ml) was stirred for 2hr at room temperature under a nitrogen atmosphere. The solvent was removed in vacuo. and the crude product recrystallised from dichloromethane/hexane to give 0.29g (67%) of the product as a white solid.

ν_{max} (nujol) 3300, 3180, 1780, 1620cm^{-1}

δ (60MHz, $CDCl_3$) 1.4 (t, 3H), 1.55 (s,9H), 3.35 (q, 2H), 9.4 (bs, 2H).

Preparation of triethyloxonium tetrafluoroborate.

Epichlorohydrin (2.38ml, 1 equiv.) was added dropwise to a stirred solution of freshly distilled boron trifluoride etherate (5.04ml, 1.3 equiv.) in sodium dried ether (25ml) under a nitrogen atmosphere. The mixture was heated at reflux for 1 hr., and allowed to stand at room temperature overnight. The supernatant ether was removed by filtration under a nitrogen atmosphere, and the crystalline solid was washed with ether (3x25ml) and dried in a stream of dry nitrogen, giving 4.92g (86%) triethyloxonium tetrafluoroborate as colourless crystals.

mp. 89°C (dec.) (lit: 91-92°C)

Preparation of 3-(p-nitrobenzyl)-[7β-phenoxyacetamido-2-aza-1-thiapenem] (343).

A solution of p-nitrobenzyl-(1-azetidin-2-onyl)-1-hydroxy-acetate (320) (360mg) in tetrahydrofuran (10ml) was stirred under a nitrogen atmosphere at -15°C. 2,6-lutidene (0.2ml, 3 equiv.) then a solution of thionyl chloride (0.08ml, 1 equiv.) in tetrahydrofuran (1ml) were added. The reaction mixture was stirred at -15°C for 3hr, then filtered. The filtrate was evaporated in vacuo. then dissolved in chloroform (3ml) and stirred under a nitrogen atmosphere at -15°C. Sodium azide (38mg, 1 equiv.) was added; the mixture was stirred for 3 hr., then washed with 5% aq. citric acid

(2x5ml), satd. sodium bicarbonate solution (5ml) and water (2x5ml), dried ($MgSO_4$) and evaporated in vacuo.
Purification by flash column chromatography on silica using 50% ethyl acetate/petrol as eluant gave 8mg product (343) (31%) as a colourless oil.
ν_{max} ($CHCl_3$ soln.) 3400, 1790, 1780, 1680cm^{-1}
δ (270MHz, $CDCl_3$) 4.57 (q, 2H), 5.53 (q, 2H), 5.52 (dd, 1H, J_1=15Hz, J_2=4Hz), 5.93 (d, 1H, J=4Hz), 6.8-8.3 (m, 9H).
m/z (+ve FAB): 409, 395, 391 (no molecular ion).

4. REFERENCES.

1. A. Fleming, Brit. J. Exp. Pathol., 1929, 10, 226.

2. Medical History, 1982, 26, 1.

3. "The Chemistry of Penicillin"; H.T. Clarke, J.R. Johnson & R. Robinson, Eds., Princeton Uni Press, 1949.

4.a) E.P. Abraham, Quart. Rev., 1967, 21, 231:
b) G. Ital. Chemioter., 1970, 17, 4:Chem. Abstr., 1972, 77, 39081.

5. "Cephalosporins & Penicillins: Chemistry & Biology"; E.H. Flynn, Ed., Academic Press, New York, 1972.

6. A.K. Mukerjee & A.K. Singh, Tetrahedron, 1978, 34, 1731.

7. P.G. Sammes, Chem. Rev., 1976, 75, 113.

8. J.H.C. Nayler, Adv. Drug Res., 1973, 7, 1.

9. P.G. Sammes, Ed., Topics in Antibiotic Chemistry, Ellis Horwood, Chichester, 1980: Vol 4. "The Chemistry and Biology of New Synthetic β-Lactam Antibiotics".

10. W. Durckheimer, J. Blumbach, R. Lattrell, & K.H. Scheunemann, Angew. Chem. Int. Ed. Engl., 1985, 24, 180.

11. J.G. Gleason & W.D. Kingsbury, Org. Cpd. S, Se & Te., 1979, 5, 454.

12. J.G. Gleason & G.L. Dunn, Org. Cpd. S, Se & Te., 1978, 4, 466.

13. J.Cs. Jaszberenyi & T.E. Gunda in Prog. Med. Chem., 12, p.395; G.P. Ellis & G.B. West, Eds., North-Holland. 1975.

14. "Recent Advances in the Chemistry of β-Lactam Antibiotics", a) Proceedings of the International Symposium, 1976: J. Elks. Ed. b) Proceedings of the Second International Symposium, 1980: G.I. Gregory. Ed. c) Proceedings of the Third International Symposium, 1984: A.G. Brown & S.M. Roberts, Eds.

15. "Chemistry and Biology of β-Lactam Antibiotics": R.B. Morin & M. Gorman, Eds., Academic Press, New York: a) Vol 1, "Penicillins and Cephalosporins": b) Vol 2, "Nontraditional β-Lactam Antibiotics": c) "The Biology of β-Lactam Antibiotics".

16. T.E. Gunda & J.Cs. Jaszberenyi in Prog. Med. Chem., 14, p.181; G.P. Ellis & G.B. West, Eds., North-Holland, 1977.

17. a) L.D. Cama & B.G. Christensen, J. Am. Chem. Soc., 1974, 96, 7582. b) idem., ibid., 1974, 96, 7584.

18. M.J. Pearson, J. Chem. Soc. Perkin Trans 1, 1981, 2544.

19. E.T. Gunda & J. Cs. Jasberenyi, Prog. Med. Chem., 1977, 14, 181.

20. J. Finkelstein, K.G. Holden, R. Sneed, & C.D. Perchonock, Tetrahedron. Lett., 1977, 22, 1855.

21.a) W. Nagata, M. Narisada & T. Yoshida in ref. 15b), p.1: b) R.D.G. Cooper in ref. 15a), p.39.

22.a) Y. Hamashima, S. Yamamoto, S. Uyeo, M. Yoshioka, M. Murakami, H. Ona, Y. Nishitani & W. Nagata, Tetrahedron Lett., 1979, 2595: b) T. Aoki, M. Yoshioka, Y. Sendo & W. Nagata, ibid., 1979, 4327: c) T. Aoki, M. Yoshioka, S. Kamata, T. Konoike, N. Haga & W. Nagata, Heterocycles, 1981, 15, 409.

23. M. Yoshioka, T. Tsuji, S. Uyeo, S. Yamamoto, T. Aoki, Y. Nishitani, S. Mori, H. Satoh, Y. Hamada, H. Ishitobi & W. Nagata, Tetrahedron Lett., 1980, 21, 351.

24. Y. Hamashima, S. Yamamoto, S. Uyeo, M. Yoshioka, M. Murakami, H. Ona, Y. Nishitani & W. Nagata, Tetrahedron Lett., 1979, 2595.

25.a) R.D.G. Cooper & F.L. Jose, J. Am. Chem. Soc., 1970, 2575: b) L.D. Hatfield, J. Fisher, F.L. Jose & R.D.G. Cooper, Tetrahedron Lett., 1970, 4897.

26. S. Uyeo, I. Kikkawa, Y. Hamashima, H. Ona, Y. Nishitani, K. Okada, T. Kubota, K. Ishikura, Y. Ide, K. Nakano & W. Nagata, J. Am. Chem. Soc., 1979, 101, 4403.

27. M. Aratani, D. Hagiwara, H. Takeno, K. Hemmi & M. Hashimoto, J. Org. Chem., 1980, 45, 3682.

28. M. Yoshioka, I. Kikkawa, T. Tsuji, Y. Nishitani, S. Mori, K. Okada, M. Murakami, F. Matsubara, M. Yamaguchi & W. Nagata, Tetrahedron Lett., 1979, 4287.

29. R. Scartazzini, H. Peter, H. Bickel, K. Heusler & R.B. Woodward, Helv. Chim. Acta., 1972, 55, 408.

30. I. Ernest, Helv. Chim. Acta., 1980, 63, 201.

31. W. Nagata, M. Narisada & T. Yoshida in ref. 15b), p.6-29.

32. S. Uyeo, I. Kikkawa, Y. Hamashima, H. Ona, Y. Nishitani, K. Okada, T. Kubota, K. Ishikura, Y. Ide, K. Nakano & W. Nagata, J. Am. Chem. Soc., 1979, 101, 4403.

33. M. Narisada, T. Yoshida, H. Onoue, M. Ohtani, K. Okada, T. Tsuji, I. Kikkawa, N. Haga, H. Satoh, H. Itani & W. Nagata, J. Med. Chem., 1979, 22, 757.

34.a) D. Habich & W. Hartwig, Tetrahedron, 1984, 40, 3667: b) S. Yamamoto, H. Itani, H. Takahashi, T. Tsuji & W. Nagata, Tetrahedron Lett., 1984, 25, 4545.

35.a) L.D. Cama & B.C. Christensen, Tetrahedron Lett., 1978, 4233: b) R.W. Ratcliffe, T.N. Salzmann & B.C. Christensen, ibid., 1980, 31.

36.a) S. Shibahara, T. Okonogi, Y. Murai, S. Fukatsu, T. Niida & T. Wakazawa, Meiji Seika Kaisha Ltd; European Patent Application 98.615 (1984): b) W. Nagata & T. Aoki, Shionogi & Co., European Patent Application 20.883 (1981).

37. S. Wolfe, J.B. Ducep, G. Kannengiesser & W.S. Lee, Can. J. Chem., 1972, 50, 2902.

38. D.H.R. Barton, M. Girijavallabhan & P.G. Sammes, J. Chem. Soc. Perkin Trans. I, 1972, 929.

39.a) Merck & Co., British Patent GB 1,455,016 (1973); Chem. Abstr. 1974, 81, 37560: b) see ref.9 p.80.

40.a) D. Davies & M.J. Pearson, J. Chem. Soc. Perkin Trans. I, 1981, 2539: b) see ref.18.

41. see ref.9 p.82.

42.a) B. Akermark, I. Lagerlund & J. Lewandowska, Acta. Chem. Scand., 1974, B28, 1238: b) E.J. Corey & A.M. Felix, J. Am. Chem. Soc., 1965, 87, 2518: c) G. Lowe & J. Parker, J. Chem. Soc. Chem. Commun., 1971, 577: d) G. Stork & R.P. Szajewski, J. Am. Chem. Soc., 1974, 96, 5787: e) D.R. Bender, L.F. Bjeldanes, D.R. Knapp & H. Rapoport. J. Org. Chem., 1975, 40, 1264: f) B. Akermark, S. Bystrom, E. Florin, N.G. Johansson & I. Lagerlund, Acta. Chem. Scand., 1974, B28, 375.

43. G. Lowe, Chemistry & Industry, 1975, 459, and refs. cited therein.

44. R.N. Guthikonda, L.D. Cama & B.C. Christensen, J. Am. Chem. Soc., 1974, 96, 7584.

45. J.A.S. Bremner, E.W. Colvin, G. Gallacher & A. MacLeod, Tetrahedron Lett., 1983, 24, 3783.

46.a) Merck & Co., British Patent GB 1,455,016 (1973); Chem. Abstr. 1974, 81, 37560: b) see ref.9 p.85.

47.a) T.W. Doyle, B. Belleau, Bing-Yu Lu, T.T. Conway, M. Menard, J.L. Douglas, D. Tim-Wu Chu, G. Lim, L.R. Morris, P. Rivest & M. Casey, Can. J. Chem., 1977, 55, 484. b) T.W. Doyle, B. Belleau, Bing Yu Luh, C.F. Ferrari & M.P. Cunningham, ibid., 1977, 55, 468: c) T.W. Doyle, T.T. Conway, M. Casey & G. Lim, ibid., 1979, 57, 222: d) T.W. Doyle, T.T. Conway, G. Lim & Bing Yu Luh, ibid., 1979, 57, 227.

48. M. Hatanaka & T. Ishimaru, Tetrahedron Lett., 1983, 24, 4837.

49. H.H. Wasserman & W.T. Han, Tetrahedron Lett., 1984, 25, 3743.

50. T.N. Salzmann, R.W. Ratcliffe, B.G. Christensen & F.A. Bouffard, J. Am. Chem. Soc., 1980, 102, 6161.

51.a) D.B. Bryan, R.F. Hall, K.G. Holden, W,F. Huffman & J.G. Gleason, J. Am. Chem. Soc., 1977, 99, 2352: b) SmithKline Corp. Belgium Patent BE 841-234 (1976).

52. see Scheme 52.

53. T.W. Doyle, J.L. Douglas, B. Belleau, J. Meunier & Bing Yu Luh, Can. J. Chem., 1977, 55, 2873.

54.a) T.W. Doyle, Bing Yu Luh & A. Martel, ibid., 1977, 55, 2700: b) T.W. Doyle, B. Belleau, Bing-Yu Lu, T.T. Conway, M. Menard, J.L. Douglas, D. Tim-Wu Chu, G. Lim, L.R. Morris, P. Rivest & M. Casey, ibid., 1977, 55, 484.

55. T.W. Doyle, Bing-Yu Luh, D.Tim-Wu Chu & B. Belleau, ibid., 1977, 55, 2719.

56. T.W. Doyle, ibid., 1977, 55, 2714.

57.a) H. Adkins & J. Reeve, J. Am. Chem. Soc., 1939, 60, 1328: b) W.G. Laver, A. Neuberger & J.J. Scott, J. Chem. Soc C, 1959, 1474.

58. J.G. Gleason, T.F. Buckley, K.G. Holden, D.B. Bryan & P. Siler, J. Am. Chem. Soc., 1979, 101, 4730.

59. T. Kametani, K. Kigasawa, M. Hiiragi, K. Wakisaka, H. Sugi & K. Tanigawa, Heterocycles, 1979, 12, 795.

60. B.H. Lee, A. Biswas & M.J. Miller, J. Org. Chem., 1986, 51, 106.

61. see Scheme 60.

62. J.G. Gleason, D.B. Bryan & K.G. Holden, Tetrahedron Lett., 1980, 21, 3947.

63.a) C.L. Branch & M.J. Pearson, J. Chem. Soc. Chem. Commun., 1981, 946: b) idem., Tetrahedron Lett., 1982, 23, 3003.

64. A. Atmani & M. Kajima, Tetrahedron Lett., 1986, 27, 2611.

65. A. Henderson, G. Johnson, K.W. Moore & B.C. Ross, J. Chem. Soc. Chem. Commun., 1982, 809.

66.a) M. Alpegiani, A. Bedeschi, E. Perrone & G. Franceschi, Tetrahedron Lett., 1984, 25, 4167: b) E. Perrone, M. Alpegiani, A. Bedeschi, M. Foglio & G. Franceschi, ibid., 1983, 24, 1631.

67.a) P.H. Crackett, C.W. Greengrass & R.J. Stoodley, J. Chem. Soc. Chem. Commun., 1983, 917: b) idem., Tetrahedron Lett., 1986, 27, 1301.

68. A.C. Kaura, C.D. Maycock & R.J. Stoodley, J. Chem. Soc. Chem. Commun., 1980, 34.

69. H.R. Pfaendler, J. Gosteli & R.B. Woodward, J. Am. Chem. Soc., 1979, 101, 6306.

70. M. Aratani & M. Hashimoto, ibid., 1980, 102, 6171.

71. C.L. Branch & M.J. Pearson, J. Chem. Soc. Perkin Trans. I, 1986, 1097.

72. C.L. Branch & M.J. Pearson, J. Chem. Soc. Perkin Trans.

I, 1979, 2268.

73.a) H.A.J. Carless & D.J. Heywood, J. Chem. Soc. Chem. Commun., 1980, 980: b) R. Boss & R. Scheffold, Angew. Chem. Int. Ed. Engl., 1976, 15, 558.

74.a) M.M. Campbell, G. Johnson, A.F. Cameron & I.R. Cameron, J. Chem. Soc. Chem. Commun., 1974, 868: b) idem., J. Chem. Soc. Perkin Trans I, 1975, 1208.

75. see a) A.W. Johnson, "Ylid Chemistry", Academic Press, New York, 1966, p.356: b) K. Tsujihara, N. Furukawa, K. Oae & S. Oae, Bull. Chem. Soc. Japan, 1969, 42, 2631.

76. C.M. Pant, R.J. Stoodley, A. Whiting & D.J. Williams, J. Chem. Soc. Chem. Commun., 1984, 1289.

77. a) G. Lowe & D.D. Ridley, J. Chem. Soc. Chem. Commun., 1973, 328. b) G. Lowe & D.D. Ridley, J. Chem. Soc. Perkin 1, 1973, 2024.

78. P.H. Bentley, P.D. Berry, G. Brooks, M.L. Gilpin, E. Hunt & I.I. Zomaya in 14b), p.175.

79. I. Ernest, J. Gosteli, C.W. Greengrass, W. Holick, D.E. Jackman, H.R. Pfaendler & R.B. Woodward, J. Am. Chem. Soc., 1978, 100, 8214.

80. R.B. Woodward in 14a), p167.

81. W.J. Laenza, F. Di Ninno, D.A. Muthard, R.R. Wilkening, K.J. Wildonger, R.W. Ratcliffe, B.G. Christensen, Tetrahedron, 1983, 39, 2505

82. T. Tanaka, T. Hashimoto, K. Iino, Y. Sugimura, T. Miyadera, J. Chem. Soc. Chem. Commun., 1982, 713.

83. a) M.D. Cooke, K.W. Moore, B.C. Ross, S.E. Turner, J. Chem. Soc. Chem. Commun. 1982, 713. b) M.D. Cooke, K.W. Moore, B.C. Ross, S.E. Turner in 3c), p100.

84. a) V.M. Girijavallabhan, A.K. Ganguly, S.W. McCombie, P. Pinto, R. Rizvi, Tetrahedron Lett., 1981, 22, 3485. b) A.K. Ganguly, V.H. Girijavallabhan, S.W. McCombie, P. Pinto, R. Rizvi, P.D. Jeffery, S. Liu, J. Antimicrob. Chemother. Suppl. C., 1982, 1.

85. G. Franceschi, M. Foglio, M. Alpegiani, C. Battistini, A. Bedeschi, E. Perrone, F. Zarini, F. Arcamone, J. Antibiot., 1983, 36, 936.

86. M.D. Cooke, K.W. Moore, B.C. Ross, S.E. Turner, J. Chem. Soc. Chem. Commun. 1983, 1005.

87. N. Daniels, G. Johnson, B.C. Ross, J. Chem. Soc. Chem. Commun. 1983, 1006.

88. V.M. Girijavallabhan, A.K. Ganguly, P. Pinto, R. Versace, J. Chem. Soc. Chem Commun., 1983, 908.

89. A. Afonso, F. Hon, J. Weinstein, A.K. Ganguly, J. Am. Chem. Soc., 1982, 104, 6138.

90. A. Yoshida, T. Hayashi, N. Takeda, S. Oida, E. Ohki, Chem. Pharm. Bull., 1983, 31, 768.

91. C. Battistini, C. Scarafile, M. Foglio, G. Franchesci, Tetrahedron Lett. 1984, 2395.

92. E. Perrone, M. Alpegiani, A. Bedeschi, F. Guidici, G. Franceschi, Tetrahedron Lett., 1985, 2399.

93. J.S. Kahan, F.M. Kahan, R. Goegelman, S.A. Currie, M. Jackson, E.O. Stapley, T.W. Miller, A.K. Miller, D. Hendlin, S. Mochales, S. Hernandez, H.B. Woodruff & J. Birnbaum, J. Antibiot., 1979, 32, 1.

94.a) A.G. Birnbaum, D. Butterworth, M. Cole, G. Hanscomb, J.D. Hood, C. Reading & G.N. Rolinson, J. Antibiot., 1976, 29, 668: b) A.G. Brown, D.F. Corbett, A.J. Eglington, T.T. Howarth, J. Chem. Soc. Chem Commun., 1977, 523: c) D.F. Corbett, A.G. Eglington & T.T. Howarth, ibid., 1977, 953: d) J.D. Hood, S.J. Box, M.S. Verrall, J. Antibiot., 1979, 32, 295: e) A.G. Brown, D.F. Corbett, A.J. Eglington & T.T. Howarth, ibid., 1979, 32, 961: f) S.J. Box, J.D. Hood & S.R. Spear, ibid., 1979, 32, 1239.

95.a) M. Nakayama, A. Iwasaki, S. Kimura, T. Mizoguchi, S. Tanabe, A. Murakami, I. Watanabe, M. Okuchi, H. Itoh, Y. Saino, F. Kobayashi & T. Mori, J. Antibiot., 1980, 33, 1388: b) Y. Nozaki, S. Harada, K.Kitano & A. Imada, ibid., 1984, 37, 218.

96.a) K. Tanaka, J. Shoji, Y. Teruji, N. Tsuji, E. Kondo, M. Mayama, Y. Kawamura, T. Hattori, K. Matsumoto & T. Yoshida, J. Antibiot., 1981, 34, 909: b) J. Shoji, H. Hinoo, R. Sakazaki, N. Tsuji, K. Nagashima, K. Matsumoto, Y. Takahashi, S. Kozuki, T. Hattori, E. Kondo & K. Tanaka, ibid., 1982, 35, 15.

97. N. Tsuji, K. Nagashima, M. Kobayashi, Y. Terui, K. Matsumoto & E. Kondo, J. Antibiot., 1982, 35, 536.

98. K. Okamura, S. Hirata, Y. Okumura, Y. Fukagawa, Y. Shimauchi, K. Kouno & T. Ishikura, J. Antibiot., 1978, 31, 480.

99. R.W. Ratcliffe & G. Albers-Schonberg in ref 14b), p.276.

100. T. Kametani, Heterocycles, 1982, 17, 463.

101.a) D.G. Melillo, I. Shinkai, T. Liu, K. Ryan & M. Sletzinger, Tetrahedron Lett., 1980, 2783: b) D.G. Melillo, T. Liu, K. Ryan, M. Sletzinger & I. Shinkai, ibid., 1981, 913.

102. K. Fujimoto, Y. Iwano & K. Hirai, Tetrahedron Lett., 1985, 26(1), 89.

103.a) K. Hirai, Y. Iwano & K. Fujimoto, Heterocycles, 1982, 17, 201: b) F. DiNinno, T.R. Beattie & B.G. Christensen, J. Org. Chem., 1977, 42(18), 2960.

104. H. Alper, C.P. Perera & F.R. Ahmed, J. Am. Chem. Soc., 1981, 103, 1289.

105. I. Nagakura, Heterocycles, 1981, 16, 1495.

106. G. Johnson, P.M. Rees & B.C. Ross, J. Chem. Soc. Chem. Commun., 1984, 970.

107. "The Chemistry of Cyanates & Their Thio Derivatives"; S. Patai, Ed., Wiley, New York, 1977; p.383-393 and refs. cited therein.

108. T. Shibata, Y. Sugimura, S. Sato & K. Kawazoe, Heterocycles, 1985, 23, 3069.

109. R. G. Alexander & R. Southgate, J. Chem. Soc. Chem. Commun., 1977, 405.

110. L.D. Cama & B.G. Christensen, Tetrahedron Lett., 1978, 4233.

111. S. Wolfe, Ger. Patent 2,356,862: Farmdoc Complete Spec. Book 34499W.

112. See ref. 16, p.188.

113.a) S. Kukolja, J. Am. Chem. Soc., 1971, 93, 6267: b) S.Kukolja & S.R. Lammert, Croat. Chim. Acta, 1972, 44, 299: c) idem, ibid, 1972, 44, 423.

114.a) B.G. Christensen & R.W. Ratcliffe, Ger. Offen 2,411,856; Chem. Abstr., 1975, 82, 31314: b) R.A. Firestone, Ger. Offen 2,411,811; Chem. Abstr., 1975, 82, 31315: c) L.D. Cama, Ger. Offen 2,501,638; Chem. Abstr., 1975, 83, 164167.

115. see ref. 9 p.55.

116. M. Alpegiani, A. Bedeschi, E. Perrone & G. Franceschi, Tetrahedron Lett., 1986, 27, 3041.

117. S. Hanessian, A. Bedeschi, C. Battistini & N. Mongelli, J. Am. Chem. Soc., 1985, 107, 1438.

118. W.F. Huffman, R.F. Hall, J.A. Grant & K.G. Holden, J. Med. Chem., 1978, 21, 413.

119. P.H. Crackett, C.M. Pant & R.J. Stoodley, J. Chem. Soc. Perkin Trans. I, 1984, 2785, and refs. cited therein.

120. J. Brennan & I.L. Pinto, Tetrahedron Lett., 1983, 24, 4731.

121. B.M. Trost & D.P. Curran, Tetrahedron Lett., 1981, 1287.

122. W.F. Huffman, K.G. Holden, T.F. Buckley, J.G. Gleason &

L. Wu, J. Am. Chem. Soc., 1977, 99, 2352.

123.a) M.J. Pearson, Tetrahedron Lett., 1982, 23, 2999: b) C.L. Branch, S.C. Finch & M.J. Pearson, ibid, 1982, 23, 4381: c) C.L. Branch & M.J. Pearson, ibid, 1983, 24, 1649: d) C.L. Branch & M.J. Pearson, J. Chem. Soc. Perkin Trans. I, 1986, 1077.

124. E.J. Moriconi & W.C. Meyer, J. Org. Chem., 1971, 36, 2841.

125. A.L. Logothetis, J. Am. Chem. Soc., 1965, 87, 749.

126.a) D.Davies & M.J. Pearson, J. Chem. Soc. Perkin Trans. I, 1981, 2544: b) C.L. Branch, S.C. Finch & M.J. Pearson, J. Chem. Soc. Perkin Trans. I, 1985, 1491: c) see ref. 18.

127.a) J. Brennan, G. Richardson & R.J. Stoodley, J. Chem. Soc. Chem. Commun., 1980, 49: b) idem, J. Chem. Soc. Perkin Trans. I, 1983, 649.

128. see Scheme 56.

129. D. Habich, P. Naab & K. Metzger, Tetrahedron Lett., 1983, 24, 2559.

130. T. Tanaka & T. Miyadera, Heterocycles, 1982, 19, 1497.

131. P.D. Jeffery & S.W. Mc.Combie, J. Org. Chem., 1982, 47, 587.

132. T. Tschamber, J.M. Henlin, D. Pipe & J. Streith, Heterocycles, 1985, 23, 2589.

133. J. Streith & T. Tschamber, Liebigs Ann. Chem., 1983,

1393.

134. P.R. Story, W.H. Morrison, T.K. Hall, K.C. Farine & C.E. Bishop, Tetrahedron Lett., 1968, 3291.

135. G. Johnson & B.C. Ross, J. Chem. Soc. Chem. Commun., 1981, 1269.

136. B.C. Ross & G. Johnson, GB 2,066,249, 1981.

137.a) R.J. Stoodley, Progr. Org. Chem., 1973, 8, 102: b) see ref. 8: c) A.K. Mukerjee & A.K. Singh, Synthesis, 1975, 547.

138. N.C. Cohen, J. Med. Chem., 1983, 26, 259.

139. C.M.D. Beels, M.S. Abu-Rabie, P. Murray-Rust & J. Murray-Rust, J. Chem. Soc. Chem. Commun., 1979, 665.

140. T. Kamiya, T. Teraji, Y. Saito, M. Hashimoto, O. Nakaguchi & T. Oku, Tetrahedron Lett., 1975, 3001.

141. M.J. Pearson, J. Chem. Soc. Perkin Trans. I, 1977, 189.

142. for example, see ref 79.

143. a) R.B. Woodward, K. Heusler, I. Ernest, K. Burri, R.J. Friary, F. Haviv, W. Oppolzer, R. Paioni, K. Syhora, R. Wenger & J.K. Whitesell, Nouveau J. Chim., 1977, 1, 85: b) K. Heusler & R.B. Woodward, Ger. Offen., 1,935,970, 1969: c) see K. Heusler in ref. 5, p273.

144. J.E. Baldwin, S.R. Herchen, J.C. Clardy, K. Hirotsu & T.S. Chou, J. Org. Chem., 1978, 43, 1342.

145. R.D.G. Cooper, P.V. Demarco & D.O. Spry, J. Am. Chem. Soc., 1969, 91, 1528.

146. a) R.B. Morin, B.G. Jackson, R.A. Mueller, E.R. Lavagnino, W.B. Scanlon & S.L. Andrews, ibid., 1969, 91, 1401: b) see ref. 7.

147. a) R.D.G. Cooper, J. Am. Chem. Soc., 1970, 92, 5010: b) D.H.R. Barton, D.G.T. Greig, G. Lucente, P.G. Sammes, M.V. Taylor, C.M. Cooper, G. Hewitt & W.G.E. Underwood, J. Chem. Soc. Chem. Commun., 1970, 1683: c) L.D. Hatfield, J. Fisher, F.L. Jose & R.D.G. Cooper, Tetrahedron Lett., 1970, 4897: d) D.H.R. Barton, P.G. Sammes, M.V. Taylor, C.M. Cooper, G. Hewitt & W.G.E. Underwood, J. Chem. Soc. Chem. Commun., 1971, 1137: e) I. Ager, D.H.R. Barton, G. Lucente & P.G. Sammes, ibid., 1972, 601.

148. see p80.

149. see Rylander, "Catalytic Hydrogenation over Platinum Metals", Academic Press, New York, 1967.

150. see, for example, a) W. Baker, C.M. Pant & R.J. Stoodley, J. Chem. Soc. Perkin Trans. I, 1978, 668: b) L.D. Cama & B.C. Christensen, J. Am. Chem. Soc., 1978, 100, 8006.

151. a) A.M. Khan, F.J. McQuillin & I. Jardine, Tetrahedron Lett., 1966, 2649: b) S. Mitsui, S. Imaizumi & Y. Esashi, Bull. Chem. Soc. Jap., 1970, 2143, and refs. cited therein.

152. O. Mitsunobu, Synthesis, 1984, 1-28.

153. I. Ernest, J. Gosteli, C.W. Greengrass, W. Holick, D.E. Jackman, H.R. Pfaendler & R.B. Woodward, J. Am. Chem. Soc., 1978, 100, 8214.

154. C.L. Branch, S.C. Finch & M.J. Pearson, J. Chem. Soc. Perkin Trans. I, 1985, 1491.

155. R.D.G. Cooper & F.L. Jose, J. Am. Chem. Soc., 1972, 94, 1021.

156. J. Blake, J.R. Tretter, G.J. Juhasz, W. Bonthrone & H. Rapoport, J. Am. Chem. Soc., 1966, 88, 4061.

157. L.A. Carpino, J. Org. Chem., 1964, 29, 2820.

158. procedure as used by Hoechst.

159. A.K. Bose & B. Lal, Tetrahedron Lett., 1973, 3937.

160. T. Mukaiyama, T. Obata & O. Mitsunobu, Bull. Chem. Soc. Jap., 1969, 38, 212.

161. S. Hanessian, D.H. Wong & M. Therien, Synthesis, 1981, 394.

162. S.R. Sandler, J. Org. Chem., 1970, 35, 3967.

163. a) J.P.H. Verheyden & J.G. Moffat, J. Org. Chem., 1972, 37, 2289: b) R. Appel, Angew. Chem. Int. Ed. Engl. 1975, 14, 801: c) J.I.G. Cadogan & R.K. Mackie, Chem. Soc. Rev., 1974, 3, 87.

164. a) R.M. Magid, O.S. Fruchey, W.L. Johnson & T.G. Allen, J. Org. Chem., 1979, 44, 359: b) R.M. Magid, O.S. Fruchey & W.L. Johnson, Tetrahedron Lett., 1977, 2999.

165. G.H. Hakimelahi & G. Just, Synthetic Communications, 1980, 10, 429.

166. M.E. Childs & W.P. Weber, J. Org. Chem., 1976, 41, 3486.

167. L.A. Carpino, J. Am. Chem. Soc., 1960, 82, 2725.

168. Y. Nii, K. Okano, S. Kobayashi & M. Ohno, Tetrahedron Lett., 1979, 2517.

169. H. Meerwein, Organic Syntheses, 1966, 46, 113.

170. H. Meerwein, P. Borner, O. Fuchs, H.J. Sasse, H. Schrodt & J. Spille, Ber., 1956, 89, 2060.

171. Y. Ito, Y. Nii, S. Kobayashi & M. Ohno, Tetrahedron Lett., 1979, 2521.

172. see refs. 63, 71, 72, 141.

173. M.S. Manhas & A.K Bose, "Synthesis of Penicillin, Cephalosporin C and Analogues", Marcel Dekker, New York (1969).

174. A.K. Bose, J. Heterocycl. Chem., 1976, 13, 93.

175. D.H.R. Barton, F. Comer, & P.G. Sammes, J. Am. Chem. Soc., 1969, 91(6), 1529.

176. D.H.R. Barton, F. Comer, D.G.T. Greig, P.G. Sammes, C.M. Cooper, G. Hewitt & W.G.E. Underwood, J. Chem. Soc. C, 1971, 3540.

177. E. Mueller & H. Huber-Emden, Ann., 1962, 660, 54.

178. Y. Maki & M. Sako, J. Am. Chem. Soc., 1977, 99(15), 5091.

179. a) Chem. Abstr., 89 163485: b) Chem. Abstr., 89 179283: c) Chem. Abstr., 89 215318: d) Chem. Abstr., 90 210189: e) Chem. Abstr., 92 198313: f) Chem. Abstr., 94 208785: g) Chem. Abstr., 95 43015: h) Chem.Abstr., 95 115359.

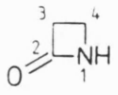
180. a) A. Balsamo, P.M. Benedini, I. Giorgi, B. Macchia & F. Macchia, Tetrahedron Lett., 1982, 2291: b) A. Balsamo, P.M. Benedini, B. Macchia, F. Macchia & A. Rossello, J.C.S. Perkin Trans. I, 1984, 413: c) D.O. Spry, J. Org. Chem., 1979, 44, 3084.

181. a) R.B. Woodward & H. Bickel, Ciba-Geigy A.G., Ger. Offen. DE 2506330, 4 Sep. 1975: b) R.B. Woodward & H. Bickel, Ciba-Geigy A.G., Ger. Offen. DE 2606196, 16 Sep. 1976: c) H. Tanida, Y. Hamashima, T. Tsuji, M. Yoshioka, M. Narisada, T. Komeno & W. Nagata, Shionogi & Co. Ltd., Japan. Kokai JP 52/7984 [77/7984], 21 Jan. 1977: d) I. Ernest, J. Gosteli & R.B. Woodward, Ciba-Geigy Corp., US 4364865A, 21 Dec. 1982.

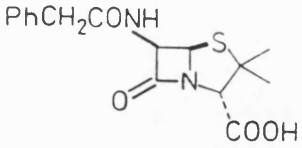
APPENDIX 1.

Nomenclature.

In the literature, monocyclic β-lactams are usually referred to as azetidin-2-ones or 2-oxoazetidines, based on the nomenclature of the parent heterocycle, azetidine. In this thesis, monocyclic β-lactam derivatives are named as azetidin-2-one derivatives (344).

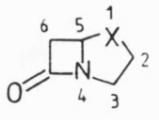


(344)

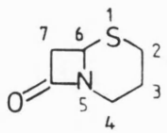


(345)

However, nomenclature of the fused β-lactams is not quite as simple. IUPAC nomenclature, although unambiguous, is somewhat unwieldy. (For example, the IUPAC name for penicillin G (345) is (6R,5R,2S) 6-benzylamido-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate)) Therefore, the trivial names "penam" and "cepham" are frequently used for the fused systems (346) and (347).[173] The hetero-analogues (348) and (349) are named as azapenams and oxapenams respectively.

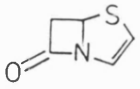


(346) X=S
(348) X=N
(349) X=O

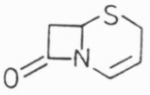


(347)

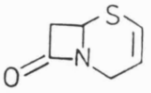
Unsaturation in the non-β-lactam ring can also be included in this system of nomenclature. Thus (350) is a penem, and (351) a cephem. Sometimes (351) is referred to as a Δ^3 cephem to distinguish it from its Δ^2 isomer.



(350)

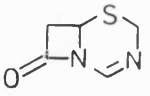


(351)

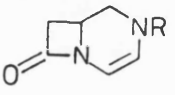


(352)

This system of trivial nomenclature can give some problems, especially in the case of fused β-lactams having no bridgehead nitrogen atom, and in those having no heteroatom at position 1 or alterations in the position of the heteroatom of the non-β-lactam ring. The latter problem can be overcome, as illustrated in the following examples. The bicyclic system (353) is referred to as a 3-azacephem, and (354) is described as a 2-aza-1-dethiacephem.



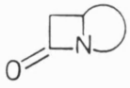
(353)



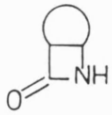
(354)

The above system of nomenclature is frequently used in the literature, and will be used throughout this thesis.

An alternative system was suggested by Bose,[174] in which fused β-lactams (355) and (356) may be called "alkanam" and "isoalkanam" respectively.



(355)



(356)

Hence β-lactams containing 7,8 & 9 atoms may be given generic names; heptanam, octanam, nonanam & so on. The numbering system conforms with the convention followed in the case of penam-cepham nomenclature. Thus the conventional penam is referred to as a 1-thiaheptam, and cepham as a 1-thiaoctanam, according to this system. Although this system was proposed 10 years ago, it has not been widely adopted.

APPENDIX 2.

Abbreviations.

Ac	acetyl
AIBN	azo-bis-isobutyronitrile
6-APA	6-aminopenicillanic acid
Ar	aryl
^{t}Bu	tert-butyl
^{t}BDMS	tert-butyldimethylsilyl
Bz	benzyl
CI	chemical ionisation
DBN	1,5-diazabicyclo[3.4.0]nonene-5
DBU	1,5-diazabicyclo[5.4.0]undecene-5
DCCI	dicyclohexylcarbodiimide
DEAD	diethylazodicarboxylate
DMAP	dimethylaminopyridine
DMB	dimethoxybenzyl
DME	1,2-dimethoxyethane
DMF	N,N-dimethylformamide
DMSO	dimethyl sulphoxide
2,4-DNP	2,4-dinitrophenylhydrazine
EEDQ	N-ethoxycarbonyl-2-ethoxy-1,2-dihydro-quinoline
EI	electron impact ionisation
Et	ethyl
FAB	fast atom bombardment
HSBT	2-mercaptobenzothiazole
HMPA	hexamethylphosphorus triamide
Im	imidazolyl
IR	infra red
LDA	lithium diisopropylamide
M^+	molecular ion
MCPBA	m-chloroperbenzoic acid
Me	methyl
MIC	minimum inhibitory concentration
m.p.	melting point

Ms	methanesulphonyl (mesyl)
NBS	N-bromosuccinimide
nmr	nuclear magnetic resonance
PCC	pyridinium chlorochromate
Ph	phenyl
PNB	p-nitrobenzyl
PNZ	p-nitrobenzyloxycarbonyl
^{i}Pr	iso-propyl
py	pyridine
SBT	S-benzothiazolyl
Tf	trifluoromethylsulphonyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSCl	trimethylsilyl chloride
TMSI	trimethylsilyl iodide
Ts	p-toluenesulphonyl (tosyl)

APPENDIX 3 - ADDITIONAL ANALYTICAL DATA.

Preparation of penicillin G sulphoxide methyl ester (285).

mp 118°C (lit[175] 128°C)
δ ($CDCl_3$) 1.11 (s, 3H, 2α-Me), 1.61 (s, 3H, 2β-Me), 3.52 (s, 2H, $PhC\underline{H}_2$), 3.72 (s, 3H, CO_2Me), 4.59 (s, 1H, 3-H), 4.94 (d, 1H, J=6Hz, 5-H), 5.93 (dd, 1H, J_1=10Hz, J_2=6Hz, 6-H), 7.23 (s, 5H, aromatics), 7.38 (d, 1H, J=10Hz, CONH).
m/z (EI) 364.02 (M^+), 229 (loss of $PhCH_2CONH$), 208 (cleavage of S1-C2, N4-C5, C6-C7 bonds), 201 (cleavage of S1-C5 and N4-C3), 190 and 174 (cleavage of C5-C6 and N4-C7), 91.

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(methyl-3'-methylbut-2'-enoate)] azetidin-2-one (286).

mp 133°C (lit[178] 138-141°C)
δ ($CDCl_3$) 1.78 (s, 3H, 3'-Me), 3.17 (s, 1H, 2'-H), 3.42 (s, 2H, $PhC\underline{H}_2$), 3.43 (s, 3H, CO_2Me), 4.70 (d, 1H, J=16Hz, $C=C\underline{H}_2$), 4.84 (d, 1H, J=16Hz $C=C\underline{H}_2$), 4.95 (dd, 1H, J_1=8Hz, J_2=3Hz, 3-H), 5.11 (d, 1H, J=3Hz, 4-H), 6.67-7.51 (m, 9H, aromatics), 8.62 (d, 1H, J=8Hz, CONH).
m/z (EI) 515.81 (M^+), 441 (loss of OMe and $CH_2=CHCH_3$), 379 (loss of $PhCH_2CONH$), 349 (loss of OMe from m/z 379) 347 (loss of SBT), 200 (cleavage of C4-S and N1-C2' bonds), 91.

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(methyl-3-oxobutanoate)]azetidin-2-one (287).

δ ($CDCl_3$) 2.22 (s, 3H, C=C-Me), 3.35 (s, 3H, CO_2Me), 3.42 (s, 2H, $PhC\underline{H}_2$), 4.74 (dd, 1H, J_1=8Hz, J_2=5Hz,

3-H), 5.19 (d, 1H, J=5Hz, 4-H), 7.1-7.6 (m, 10H, aromatics), 10.8 (s, 1H, CONH).
m/z (+ve FAB) 516.81 (M^+), 411, 382 (loss of $PhCH_2CONH$), 349 (loss of SSBT), 289 (loss of CO from m/z 317), 91.

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(methyl-3'-methanesulphonylbut-2'-enoate)] azetidin-2-one (288).

δ ($CDCl_3$) 1.22 (s, C=CMe, minor isomer), 2.04 (s, C=CMe, major isomer), 2.59 (s, OMs, minor isomer), 3.28 (s, OMs, major isomer), 3.59 (s, 2H, $PhC\underline{H}_2$), 3.66 (s, CO_2Me, major isomer), 3.81 (s, CO_2Me, minor isomer), 5.12 (dd, 1H, J_1=8Hz, J_2=4.5Hz, 3-H), 5.61 (d, 1H, J=4.5Hz, 4-H), 7.20-8.00 (m, 10H, aromatics and CONH).
m/z (+ve FAB) 594.93 (M^+), 489, 475 (loss of $PhCH_2CO$), 428 (loss of SBT), 395 (loss of S from m/z 428), 314, 200 (cleavage of C4-S and N1-C2' bonds), 91.

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(methyl-3'-phenylamidobut-2'-enoate)] azetidin-2-one (289).

δ ($CDCl_3$) 1.23 (s, C=CMe, minor isomer), 2.41 (s, C=CMe, major isomer), 3.47 (s, 2H, $PhC\underline{H}_2$), 3.61 (s, CO_2Me, major isomer), 3.66 (s, CO_2Me, minor isomer), 4.59 (dd, 1H, J_1=7Hz, J_2=5Hz, 3-H) 5.44 (d, 1H, J=5Hz, 4-H), 6.92-7.32 (m, 9H, aromatics), 8.83 (d, 1H, J=7Hz, CONH).
m/z (+ve FAB) 592.05 (M^+), 486, 454 (loss of OMe from m/z 486), 424 (loss of SBT), 392 (loss of SSBT), 361 (loss of OMe from m/z 392), 200 (cleavage of C4-S and N1-C2' bonds), 91.

Preparation of methyl[2-phenyl-7β-phenylacetamido-2-aza cephalosporanate] (290).

δ ($CDCl_3$) 2.12 (s, 3H, 3-Me), 3.51 (s, 2H, $PhC\underline{H}_2$), 3.79 (s, 3H, CO_2Me), 4.76 (d, 1H, J=5Hz, 6-H), 5.89 (d, 1H, J=5Hz, 7-H: and bs, 1H, CONH), 6.82-7.37 (m, 10H, aromatics). The resonance at 5.89 due to the amide proton is eliminated by deuterium exchange.

m/z (high res. EI) 423.1245 (M^+). $C_{22}H_{21}N_3O_4S$ requires 423.1253. Fragmentations: 331 (cleavage of S1-N2 and N2-C3 bonds), 272 (cleavage of S1-C6 and C3-C4), 264, 249 (cleavage of C6-C7 and C8-N5 bonds), 247 (due to $[PhCH_2CONHCH_2CH{=}N{=}CO_2Me]^+$), 233 (cleavage of S1-N2 and N5-C4), 217 (loss of MeOH from m/z 249), 200 (cleavage of S1-C6 and C4-N5), 91.

analysis found C 62.1%, H 5.0%, N 9.1%
calc. C 62.4%, H 5.0%, N 9.9%

Preparation of penicillin G sulphoxide p-nitrobenzyl ester.

mp (ethyl acetate/petrol) 140°C (lit[176] 142-144°C)
δ ($CDCl_3$) 1.10 (s, 3H, 2α-Me), 1.64 (s, 3H, 2β-Me), 3.52 (s, 2H, $PhC\underline{H}_2$), 4.65 (s, 1H, 3-H), 4.93 (d, 1H, J=4Hz, 5-H), 5.25 (s, 2H, $C\underline{H}_2C_6H_4NO_2$), 5.95 (dd, 1H, J_1=4Hz, J_2=10Hz, 6-H), 7.1-8.2 (m, 10H, aromatics and CONH).

m/z (high res EI) 485.5163 (M^+). $C_{23}H_{23}N_3O_7S$ requires 485.5097.

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-methylbut-2'-enoate)] azetidin-2-one.

mp 153°C (Searching of Chemical Abstracts revealed no references to the mp. of this compound.)
δ ($CDCl_3$) 1.95 (s, 3H, 3'-Me), 3.71 (s, 2H, $PhC\underline{H}_2$), 5.04 (s, 2H, $C\underline{H}_2C_6H_4NO_2$), 5.14 (s, 1H, 2'-H), 5.18 (d, 1H, J=3Hz, 3-H), 5.31 (d, 1H, J=3Hz, C=C-Me), 5.39 (d,

1H, J=3Hz, C=C-Me) 5.43 (dd, 1H, J_1=3Hz, J_2=11Hz, 4-H), 7.2-8.3 (m, 14H, aromatics and CONH).

m/z Difficulties were encountered in obtaining a satisfactory mass spectrum. No peaks were observed in either +ve or -ve FAB, and a molecular ion was obtained in neither EI nor CI. Fragmentations: (70eV EI) 332 (loss of SBT and PNB), 268. (CI) 297, 273 (loss of OPNB then cleavage of N1-C4 and C3-C4), 219 ($[H_2C{=}C{=}CHCO_2PNB]^+$), 203, 202, 196, 190, 136 ($[O_2NC_6H_4CH_2]^+$).

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3-oxobutanoate)] (292)

δ ($CDCl_3$) 2.30 (s, 3H, C=C-Me), 3.52 (s, 2H, $PhC\underline{H}_2$), 5.05 (d, 1H, J=4Hz, 4-H), 5.20 (s, 2H, $C\underline{H}_2C_6H_4NO_2$), 5.30 (dd, 1H, J_1=4Hz, J_2=9Hz, 3-H), 6.8-7.9 (m, 13H, aromatics and CONH), 11.8 (s, 1H, C=C-OH)

m/z Difficulties were encountered in obtaining a satisfactory mass spectrum. No peaks were observed in either +ve or -ve FAB, and a molecular ion was obtained in neither EI nor CI. Fragmentations: (70eV EI) 247, 359 (corresponding to the fragment $[PhCH_2CONHCH{=}CHS\text{-}SBT]^+$), 268 (loss of SBT, OPNB and H_2O), 226, 206, 151, 105, 91.

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-phenylamidobut-2'-enoate)] azetidin-2-one.

δ (60 MHz, $CDCl_3$, major isomer) 2.38 (s, 3H, C=C-Me), 3.51 (s, 2H, $PhC\underline{H}_2$), 4.78 (dd, 1H, J_1=9Hz, J_2=5Hz, 3-H), 4.94 (s, 2H, $C\underline{H}_2C_6H_4NO_2$), 5.02 (bs, 1H, $N\underline{H}Ph$), 5.38 (d, 1H, J=5Hz, 4-H), 6.64-7.99 (m, 18H, aromatics), 9.1 (bs, 1H, CONH). The resonance at 5.02ppm was removed by deuterium exchange.

m/z Due to the reactivity of this compound it was not possible to obtain a molecular ion via any ionisation

technique. However, with +ve FAB, fragmentations were observed at 545 (loss of SBT), 512 (loss of SSBT), 393 (loss of OPNB from m/z 544), 365 (loss of CO from m/z 393), 353, 333, 332, 275, 247, 230, 215, 214, 213, 201, 185, 167, 118, 91.

analysis Satisfactory analytical data could not be obtained for this compound, due to its instability.

Preparation of 4-nitrobenzyl[2-phenyl-7β-phenylacetamido-2-aza-1-thiacephalosporanate] (293).

δ (60MHz, d⁶ DMSO) 2.09 (s, 3H, 3-Me), 3.44 (s, 2H, $PhC\underline{H}_2$), 4.89 (d, 1H, J=7Hz, 6-H), 5.28 (s, 2H, $C\underline{H}_2C_6H_4NO_2$), 5.85 (dd, 1H, J_1=7Hz, J_2=9Hz, 7-H), 6.86-8.17 (m, 14H, aromatics), 8.82 (d, 1H, J=9Hz, CONH).

analysis found C 61.2%, H 4.2%, N 9.5%
calc. C 62.6%, H 4.3%, N 10.0%

N.B. A very small quantity of sample was available for the analysis, consequently a greater degree of experimental error has been introduced.

Preparation of potassium [2-phenyl-7β-phenylacetamido-2-aza-1-thiacephalosporanate] (294).

m.p. (ethyl acetate) 187° C.

δ (D_2O, 250MHz) 1.63 (s, 3H, 3-Me), 4.78 (d, 1H, J=11Hz, $PhC\underline{H}_A$), 4.85 (d, 1H, J=11Hz, $PhC\underline{H}_B$), 5.33 (d, 1H, J=4.5Hz, 6-H), 5.96 (d, 1H, J=4.5Hz, 7-H), 7.0-7.4 (m, 10H, aromatics). The resonance due to the amide proton was not observed due to deuterium exchange.

m/z A molecular ion was not observed for this compound. This is attributed to its lack of stability. The following fragmentations were observed: (-ve FAB) m/z 363 ($M^+ - CO_2$), 233 (cleavage of N5-C8 and C6-C7, and loss of CO_2): (+ve FAB) m/z 289 (loss of $PhCH_2CO$) .

analysis Difficulty was experienced in obtaining an elemental analysis of this compound, due to lack of material and its instability.

Preparation of 3-(phenylacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'(4-nitrobenzyl-3'-methylamidobut-2'-enoate)] azetidin-2-one.

δ (60MHz, $CDCl_3$) major isomer 2.28 (s, 3H, C=C-Me), 2.86 (s, 3H, NMe), 3.57 (s, 2H, $PhC\underline{H}_2$), 4.87 (dd, 1H, J_1=7Hz, J_2=10Hz, 3-H), 5.09 (bs, 1H, N<u>H</u>Me), 5.23 (d, 1H, J=7Hz, 4-H), 6.98-8.14 (m, 13H, aromatics), 8.94 (d, J=10Hz, CONH).
Satisfactory mass spectral and elemental analysis data were not obtained for this compound, due to its lack of stability.

Preparation of 4-nitrobenzyl [2-methyl-7β-phenylacetamido-2-azacephalosporanate] (299).

δ (60MHz, d⁶ DMSO) 2.30 (s, 3H, 3-Me), 3.26 (s, 3H, 2-Me), 3.43 (s, 2H, $PhC\underline{H}_2$), 4.91 (d, 1H, J=6Hz, 6-H), 5.18 (s, 2H, $C\underline{H}_2C_6H_4NO_2$), 5.81 (dd, 1H, J_1=11Hz, J_2=6Hz, 7-H), 6.97-8.16 (m, 9H, aromatics), 8.78 (d, 1H, J=11Hz, CONH).
Satisfactory mass spectral and elemental analysis data were not obtained for this compound, due to its lack of stability.

Preparation of penicillin V sulphoxide p-nitrobenzyl ester.

mp 148°C (A search of Chemical Abstracts revealed several references[179] to this compound in the literature, however a mp. was not quoted in any of these, nor were references to previous preparations of this compound cited.)
δ ($CDCl_3$) 1.16 (s, 3H, 2α-Me), 1.72 (s, 3H, 2β-Me), 4.54 (s, 2H, $PhOC\underline{H}_2$), 4.75 (s, 1H, 3-H), 5.05 (d, 1H, J=5Hz, 5-H), 5.34 (d, 1H, J=15Hz, $C\underline{H}_AC_6H_4NO_2$), 5.41 (d, 1H, J=15Hz, $C\underline{H}_BC_6H_4NO_2$), 6.13 (dd, 1H, J_1=5Hz, J_2=10Hz, 6-H),

6.9-8.3 (m, 10H, aromatics and CONH).
m/z (high res EI) 501.4997 (M^+). $C_{23}H_{23}N_3O_8S$ requires 501.5091.

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-methyl-but-2'-enoate)]azetidin-2-one (308).

mp 139-142°C (A search of Chemical Abstracts revealed several references[180] to this compound in the literature. However, none of these references quoted a value for its mp. Several references[181] to this compound in the patent literature were also obtained. Unfortunately, at the time of writing the author had no access to the patent literature.)
δ (d⁶DMSO) 1.98 (s, 3H, 3'-Me), 4.52 (d, 1H, J=15Hz, $PhOCH_AH_B$), 4.62 (d, 1H, J=15Hz, $PhOCH_AH_B$), 5.02 (d, 2H, J=3Hz, $C=CH_2$), 5.19 (d, 1H, J=11Hz, $CH_AH_BC_6H_4NO_2$), 5.26 (d, 1H, J=11Hz, $CH_AH_BC_6H_4NO_2$), 5.24 (s, 1H, 2'-H), 5.47 (dd, 1H, J_1=5Hz, J_2=9Hz, 3-H), 5.56 (d, 1H, J=5Hz, 4-H), 6.9-8.2 (m, 14H, aromatics and CONH).
m/z Molecular ion not observed with +ve or -ve FAB, EI or CI. Fragmentations: (+ve FAB) 551 (loss of $PhOCH_2$), 499 (loss of OPNB), 485 (loss of SBT), 471 (loss of CO_2PNB), 460 (cleavage of N1-C2 and C3-C4), 455 (loss of OPNB and $H_2C=CHCH_3$), 453 (loss of SSBT), 391, 337, 278 (cleavage of N1-C4 and C2-C3), 207, 168.

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(4-nitrobenzyl)-3'-oxobutanoate))]-azetidin-2-one (295).

δ (250MHz, $CDCl_3$) 2.38 (s, 3H, C=C-Me), 4.58 (d, 1H, J=12Hz, $PhOCH_AH_B$), 4.66 (d, 1H, J=12Hz, $PhOCH_AH_B$), 5.10 (d, 1H, J=13Hz, $CH_AH_BC_6H_4NO_2$), 5.24 (d, 1H, J=13Hz, $CH_AH_BC_6H_4NO_2$), 5.19 (dd, 1H, J_1=5.2Hz, J_2=10.4Hz, 3-H), 5.41 (d, 1H, J=5.0Hz, 4-H), 6.93-8.05 (m, 13H, aromatics), 7.40 (dd, 1H, J_1=10.6Hz,

J_2 =7.5Hz, CONH), 12.21 (s, 1H, C=C-OH).

m/z Molecular ion not observed with +ve or -ve FAB, EI or CI. Fragmentations: (+ve FAB) 485 (loss of HSBT), 454 (loss of SSBT), 390, 375 (cleavage of C4-N1 and C2-C3), 370, 368, 336 (loss of SBT and $PhOCH_2CONH$), 344 (loss of SBT and OPNB), 305 (loss of SSBT and $PhOCH_2CONH$), 302 (loss of SSBT and OPNB).

analysis found C 53.5%, H 3.8%, N 8.4%

calc. C 53.4%, H 3.7%, N 8.6%

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-methanesulphonylbut-2'-enoate))]azetidin-2-one.

δ (250MHz, $CDCl_3$) major isomer: 2.59 (s, 3H, C=C-Me), 3.19 (s, 3H, OSO_2Me), 4.59 (d, 1H, J=12Hz, $PhOCH_AH_B$), 4.67 (d, 1H, J=12Hz, $PhOCH_AH_B$), 5.00 (d, 1H, J=15Hz, $CH_AH_BC_6H_4NO_2$), 5.10 (dd, 1H, J_1=5Hz, J_2=8Hz, 3-H), 5.12 (d, 1H, J=15Hz, $CH_AH_BC_6H_4NO_2$), 5.61 (d, 1H, J=5Hz, 4-H), 6.2-8.2 (m, 14H, aromatics and CONH).

Difficulty was encountered in obtaining a satisfactory mass spectrum of this compound. No peaks were observed with either +ve or -ve FAB. Due to the lack of stability of the compound a molecular ion was observed in neither EI or CI.

It was not possible to obtain elemental analysis of this compound, as separation of this compound and its starting material proved impossible.

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-(4-toluenesulphonyl)but-2'-enoate))]azetidin-2-one (297).

δ (250MHz, $CDCl_3$) major isomer: 1.60 (s, 3H, p-C_6H_4Me), 2.49 (s, 3H, C=C-Me), 4.50 (d, 1H, J=13Hz, $PhOCH_AH_B$), 4.58 (d, 1H, J=13Hz, $PhOCH_AH_B$), 5.19 (d, 1H, J=15Hz, $CH_AH_BC_6H_4NO_2$), 5.31 (d, 1H, J=15Hz, $CH_AH_BC_6H_4NO_2$), 5.58 (dd, 1H, J_1=5Hz,

J_2=9Hz, 3-H), 5.70 (d, 1H, J=5Hz, 4-H), 6.8-8.3 (m, 18H, aromatics and CONH).

Difficulty was encountered in obtaining a satisfactory mass spectrum of this compound. No peaks were observed with either +ve or -ve FAB. Due to the lack of stability of the compound a molecular ion was observed in neither EI or CI.

Fragmentations were observed as follows: (70eV EI) 332, 268, 167, 119, 105, 91, (CI) 269, 268, 228, 168, 167.

It was not possible to obtain elemental analysis of this compound, as separation of this compound and its starting material proved impossible.

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-trifluoromethanesulphonylbut-2'-enoate)]azetidin-2-one (298).

δ ($CDCl_3$) major isomer: 2.55 (s, 3H, C=C-Me), 4.61 (d, 1H, J=11Hz, PhOC$\underline{H}_A$), 4.68 (d, 1H, J=11Hz, PhOC$\underline{H}_B$), 4.96 (d, 1H, J=14Hz, C$\underline{H}_A$$C_6H_4NO_2$), 5.09 (d, 1H, J=14Hz, C$\underline{H}_B$$C_6H_4NO_2$), 5.10 (dd, 1H, J_1=5.2Hz, J_2=2.2Hz, 3-H), 5.73 (d, 1H, J=5.5Hz, 4-H), 6.89-8.18 (m, 13H, aromatics), 7.42 (dd, 1H, J_1=2.1Hz, J_2=7.0Hz, CONH).

analysis found C 46.2%, H 3.1%, N 6.9%

calc. C 47.9%, H 3.1%, N 7.4%

Some difficulty was encountered in obtaining a satisfactory analysis for this compound, due to its lack of stability.

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(4-nitrobenzyl)-3'-phenylamidobut-2'-enoate)]azetidinone (296, R=Ph).

δ ($CDCl_3$) 2.45 (s, 3H, C=C-Me), 4.60 (d, 1H, J=11Hz, PhOC$\underline{H}_A$), 4.67 (d, 1H, J=11Hz, PhOC$\underline{H}_B$), 4.85 (dd, 1H, J_1=5Hz, J_2=8Hz, 3-H), 5.17 (d, 1H, J=14Hz, C$\underline{H}_A$$C_6H_4NO_2$), 5.26 (d, 1H, J=14Hz, C$\underline{H}_B$$C_6H_4NO_2$), 5.53 (d, 1H, J=5Hz, 4-H), 6.9-8.1 (m, 19H, aromatics and CONH), 8.98 (s, 1H, N$\underline{H}$Ph).

analysis found C 56.9%, H 3.8%, N 9.1%
calc. C 57.8%, H 3.9%, N 9.6%

Preparation of 4-nitrobenzyl[2-phenyl-7β-phenoxyacetamido-2-azacephalosporanate] (300).

δ (270MHz, d⁶ DMSO) 2.11 (s, 3H, 3-Me), 4.58 (s, 2H, $PhOC\underline{H}_2$), 5.05 (d, 1H, J=5Hz, 7-H), 5.41 (s, 2H, $C\underline{H}_2C_6H_4NO_2$), 6.06 (dd, 1H, J_1=5Hz, J_2=8Hz, 6-H), 6.8-7.4 (m, 10H, $C_6\underline{H}_5O$ and $NC_6\underline{H}_5$), 7.75 (d, 2H, J=9Hz, $CH_2C_6\underline{H}_4NO_2$, protons m- to nitro substituent), 8.25 (d, 2H, J=9Hz, $CH_2C_6\underline{H}_4NO_2$, protons o- to nitro substituent), 9.11 (d, 1H, J=8Hz, CONH). As noted in the Experimental, difficulties were encountered in obtaining a satisfactory mass spectrum of this compound. Some fragment ions were observed with 70eV EI. Some of these were assigned in the Experimental. Other fragments observed are as follows: 408, 353, 151, 136, 107, 93, 77.

analysis found C 59.4%, H 4.3%, N 9.6%
calc. C 60.8%, H 4.2%, N 9.8%

N.B. A very small quantity of sample was available for the analysis, consequently a greater degree of experimental error has been introduced.

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(4-nitrobenzyl)-3'-cyanomethylamidobut-2'-enoate)]azetidinone. (296, R=CH_2CN)

δ ($CDCl_3$) major isomer: 2.60 (s, 3H, C=C-Me), 4.18 (d, 1H, J=8Hz, $NC\underline{H}_ACN$), 4.24 (d, 1H, J=8Hz, $NC\underline{H}_BCN$), 4.57 (d, 1H, J=13Hz, $PhOC\underline{H}_A$), 4.64 (d, 1H, J=13Hz, $PhOC\underline{H}_B$), 4.81 (dd, 1H, J_1=4.8Hz, J_2=2.9Hz, 3-H), 5.01 (d, 1H, J=18Hz, $C\underline{H}_AC_6H_4NO_2$), 5.10 (d, 1H, J=18Hz, $C\underline{H}_BC_6H_4NO_2$), 5.46 (d, 1H, J=4.8 Hz, 4-H), 6.94-8.04 (m, 13H, aromatics), 8.22 (dd, 1H, J_1=5.5Hz, J_2=2.9Hz, CONH), 8.74 (t, 1H, J=8Hz, $N\underline{H}CH_2CN$). The resonance

at 8.74 ppm was removed by deuterium exchange.
m/z No molecular ion was observed with either +ve or -ve FAB, or with EI or CI. This is thought to be due to the lack of stability of this compound. Some fragment ions were noted with +ve FAB, some of these have been assigned in the Experimental. Other fragments noted with +ve FAB are as follows: m/z 245, 207, 191, 153, 115, 99.
analysis found C 53.5%, H 4.0%, N 12.1%
calc. C 53.9%, H 3.8%, N 12.2%

Preparation of 4-nitrobenzyl[2-cyanomethyl-7β-phenoxy-acetamido-2-azacephalosporanate] (301).

δ (400MHz, $CDCl_3$) 2.41 (s, 3H, 3-Me), 4.20 (d, 1H, J=18Hz, NCH_ACN), 4.26 (d, 1H, J=18Hz, NCH_BCN), 4.54 (s, 2H, $PhOCH_2$), 5.16 (d, 1H, J=4.6Hz, 7-H), 5.31 (d, 1H, J=14Hz, $CH_AC_6H_4NO_2$), 5.38 (d, 1H, J=14Hz, $CH_BC_6H_4NO_2$), 6.13 (dd, 1H, J_1=4.6Hz, J_2=3.6Hz, 6-H), 7.04 (dd, 1H, J_1=3.6Hz, J_2=7.4Hz, CONH) 6.88-7.40 (m, 5H, C_6H_5O), 7.62 (d, 2H, J=8Hz, $CH_2C_6H_4NO_2$, protons m- to nitro substituent) 8.24 (d, 2H, J=8Hz, $CH_2C_6H_4NO_2$, protons o- to nitro substituent).
m/z No molecular ion was observed with either +ve or -ve FAB, or with EI or CI. This is thought to be due to the lack of stability of this compound. Some fragment ions were noted with +ve FAB, which have been assigned in the Experimental.
analysis found C 54.7%, H 3.9%, N 12.9%
calc. C 55.1%, H 4.0%, N 13.4%

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-((4-nitrobenzyl)-3'-methylbut-3'-enoate)] azetidinone (309).

mp (toluene) 113°C (lit[153] 115°C).
δ ($CDCl_3$ soln.) 2.18 (s, 3H, C=C-Me), 2.23 (s, 3H, C=C-Me), 4.55 (d, 1H, J=11Hz, $PhOCH_A$), 4.62 (d, 1H, J=11Hz,

PhOC$\underline{H}_B$), 5.01 (d, 1H, J=11Hz, C$\underline{H}_A$C$_6$H$_4$NO$_2$), 5.12 (dd, 1H, J_1=8Hz, J_2=5Hz, 3-H), 5.15 (d, 1H, J=11Hz, C$\underline{H}_B$C$_6$H$_4$NO$_2$), 5.50 (d, 1H, J=5Hz, 4-H), 6.8-8.0 (m, 14H, aromatics).
m/z No molecular ion was observed with either +ve or -ve FAB, or with EI or CI. Some fragment ions were noted with +ve FAB: m/z 551 (loss of PhOCH$_2$), 485 (loss of SBT), 459 (cleavage of N1-C2 and C3-C4), 453 (loss of SSBT), 334 (loss of CO$_2$PNB and PhOCH$_2$CONH).

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl) azetidinone (305).

mp. 152-154°C (lit[153] 156-158°C)
δ (d^{8}THF) 4.56 (d, 1H, J=15Hz, PhOC$\underline{H}_A$), 4.64 (d, 1H, J=15Hz, PhOC$\underline{H}_B$), 5.31 (d, 1H, J=5Hz, 4-H), 5.52 (dd, 1H, J_1=9Hz, J_2=5Hz, 3-H), 6.9-7.9 (m, 9H, aromatics), 8.23 (s, 1H, 1-H), 8.48 (d, 1H, J=9Hz, CONH).
m/z (+ve FAB) 418 (M^++1), 391 (loss of CO from β-lactam ring), 386 (loss of S), 375 (loss of CONH), 311 (loss of PhOCH$_2$), 252 (loss of SBT), 220 (loss of SSBT), 192 and 378 (arising from cleavage of N1-C2 and C3-C4).
analysis found C 51.4%, H 3.6%, N 9.8%
calc. C 51.8%, H 3.6%, N 10.0%

Preparation of di-t-butyl fumarate (315).

mp. 65-67°C (lit[177] 69-70°C).
δ ($CDCl_3$, 60MHz) 1.45 (s, 9H, CO$_2^{t}\underline{Bu}$), 6.60 (s, 1H, C=C$\underline{H}$).
m/z (CI) 229 (M^++H), 173 (M^+-(t-Bu)).

Preparation of di-t-butyl tartrate (316).

mp. 82-83°C (lit[156] 84-85°C).
δ ($CDCl_3$, 60MHz) 1.50 (s, 18H, CO$_2^{t}\underline{Bu}$), 3.15 (d, 2H, -O$\underline{H}$), 4.40 (d, 2H, C$\underline{H}$OH).
m/z (CI) 263 (M^++H).

Preparation of t-butyl glyoxylate monohydrate (317).[155]

δ ($CDCl_3$ soln.) 1.15 (s, 9H, $CO_2{}^tBu$), 3.64 (m, 1H, -OH), 3.80 (m, 1H, -OH), 5.16 (dd, 1H, J_1=9Hz, J_2=3Hz, $CH(OH)_2$). N.B. The two hydrate protons are non-equivalent.
m/z (CI) 149 (M^++H).

Preparation of di-(4-nitrobenzyl) tartrate (318).

δ (60MHz, d⁶ DMSO) 5.30 (s, 2H, $CH_2C_6H_4NO_2$), 5.85 (bs, 2H, -OH), 7.60 (d, 2H, J=8Hz, $CH_2C_6H_4NO_2$, protons m- to nitro substituent), 8.10 (d, 2H, J=8Hz, $CH_2C_6H_4NO_2$, protons o- to nitro substituent).

Preparation of 4-nitrobenzyl glyoxylate monohydrate (319).

δ (60MHz, d⁶ DMSO) 3.35 (dd, 1H, J_1=7Hz, J_2=2Hz, -OH), 4.65 (dd, 1H, J_1=7Hz, J_2=2Hz, -OH), 5.30 (s, 2H, $CH_2C_6H_4NO_2$), 5.78 (s, 1H, $CH(OH)_2$), 7.60 (d, 2H, J=8Hz, $CH_2C_6H_4NO_2$, protons m- to nitro substituent), 8.16 (d, 2H, J=8Hz, $CH_2C_6H_4NO_2$, protons o- to nitro substituent).
m/z (70eV EI) 227 (M^+), 209 (loss of H_2O), 91 (loss of PNB).

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(p-nitrobenzyl)-2'-hydroxyacetate)] azetidin-2-one (320).

δ ($CDCl_3$) - product was obtained as a diastereomeric mixture: 4.71 (m, 2H, $PhOCH_2$), 5.32 (m, 2H, $CH_2C_6H_4NO_2$), 5.38 (dd, 1H, J_1=5Hz, J_2=8Hz, 3-H), 5.48 (d, 1H, J=5Hz, 4-H), 5.70 (s, 1H, 2'-H), 6.86 (bs, 1H, -OH), 7.0-8.3 (m, 13H, aromatics), 9.28 (dd, 1H, J_1=8Hz, J_2=8Hz, CONH).

m/z (+ve FAB) 627 ($M^+ +1$), 609 (loss of H_2O), 534 (loss of PhO), 475 (loss of OPNB), 461 (loss of SBT), 429 (loss of SSBT).

analysis found C 51.2%, H 3.7%, N 8.4%
calc. C 50.5%, H 3.5%, N 8.7%

Preparation of 3-(phenoxyacetamido)-4-(2'-dithiobenzothiazolyl)-1-[2'-(t-butyl)-2'-hydroxyacetate)] azetidin-2-one (321).

δ ($CDCl_3$) - product was obtained as a diastereomeric mixture: 1.30 and 1.49 (two singlets, due to ^tBu group of each enantiomer, total 9H, ^tBu), 4.57 (m, 2H, $PhOC\underline{H}_2$), 5.28 (s, 1H, 2'-H), 5.50 (m, 2H, $C\underline{H}_2C_6H_4NO_2$), 5.60 (d, 1H, J=8Hz, 4-H), 5.64 (dd, 1H, J_1=5Hz, J_2=8Hz, 3-H), 6.9-8.0 (m, 10H, aromatics and CONH), 9.28 (s, 1H, -O<u>H</u>).

m/z (+ve FAB) 548 ($M^+ +1$), 530 (loss of H_2O), 491 (loss of ^tBu), 455 (loss of PhO), 382 (loss of SBT), 350 (loss of SSBT).

analysis found C 52.2% H 4.3% N 7.8%
calc. C 52.6% H 4.6% N 7.7%

Preparation of p-nitrobenzyl(2-benzyl-6β-phenoxyacetamido-2-aza-1-thiapenicillanate) (322).

(322)

δ ($CDCl_3$) - product was obtained as a diastereomeric mixture: 4.47 (d, 1H, J=15Hz, $PhOC\underline{H}_A$), 4.58 (d, 1H, J=15Hz, $PhOC\underline{H}_B$), 5.21 (s, 2H, $NC\underline{H}_2Ph$), 5.35 (d, 1H, J=13Hz, $C\underline{H}_AC_6H_4NO_2$), 5.49 (d, 1H, J=13Hz, $C\underline{H}_BC_6H_4NO_2$), 5.61 (dd, 1H, J_1=5Hz, J_2=8Hz, 6-H), 5.82 (d, 1H, J=5Hz, 5-H), 6.65 (two singlets, total 1H, mixture of 3α-H and 3β-H), 6.8-8.2 (m, 15H). m/z (+ve FAB) 549 ($M^+ +1$), 458 (loss of $PhCH_2$), 443 (loss of $PhCH_2NH$), 413 (loss of PNB), 397 (loss of OPNB).

Difficulty was encountered in obtaining a satisfactory analysis for this compound, due to its lack of stability.

(323)

δ ($CDCl_3$) - product was obtained as a diastereomeric mixture, consequently it was not possible to determine the coupling constants for some protons: 4.04 (m, 2H, $NHC\underline{H}_2Ph$), 4.56 (m, 2H, $PhOC\underline{H}_2$), 4.83 (s, 1H, $C\underline{H}CO_2PNB$), 5.13 (m, 2H, $C\underline{H}_2C_6H_4NO_2$), 5.24 (dd, 1H, J_1=5Hz, J_2=7Hz, 3-H), 5.32 (d, 1H, J=5Hz, 4-H), 6.9-8.0 (m, 18H, aromatics), 8.06 (d, 1H, CONH), 8.20 (m, 1H, $N\underline{H}CH_2Ph$).

m/z (+ve FAB) 716 (M^++1), 625 (loss of $PhCH_2$), 609 (loss of $PhCH_2NH_2$), 548 (loss of HSBT), 535 (loss of CO_2PNB), 475 (loss of PNB and $PhCH_2NH_2$), 459 (loss of SBT from m/z 625).

Difficulty was encountered in obtaining a satisfactory analysis for this compound, due to its lack of stability.

Preparation of t-butyl(1-benzyliminoacetate) (306, R'=Bz, R"=^{t}Bu.

lit[165] - oil, no b.p. given. IR (lit[165]) 1735, 1710, 1640cm^{-1}

δ (lit[165]) 1.6 (s, 9H), 4.9 (d, 2H, J=1Hz), 7.39 (s, 5H), 7.63 (m, 1H).

δ ($CDCl_3$ soln.) 1.54 (s, 9H, ^{t}Bu), 4.83 (s, 2H, $C\underline{H}_2Ph$), 7.20-7.30 (m, 5H, aromatics), 7.63 (s, 1H, $\underline{H}C{=}N$).

Preparation of 4-nitrobenzyl(1-benzyliminoacetate) (306, R'=Bz, R"=PNB).

A mixture of benzylamine (0.26g, 1.1 equiv.), dichloromethane (10ml) and activated molecular sieves was stirred at 0°C under a nitrogen atmosphere. 4-Nitrobenzyl glyoxylate (317)

(0.50g, 1 equiv.) in dichloromethane (10ml) was added with stirring over a period of 20 min. The mixture was stirred at room temperature for a further 90 min, then filtered and evaporated <u>in vacuo</u>. Purification by flash column chromatography on silica gel deactivated with 10% water gave the imine (306, R'=Bz, R"=PNB) (0.34g, 54%) as a pale yellow oil.

ν_{max} 1720, 1640cm^{-1}
δ ($CDCl_3$, 60MHz) 4.90 (d, 2H, J=2Hz, $C\underline{H}_2Ph$), 5.36 (s, 2H, $C\underline{H}_2C_6H_4NO_2$), 7.40 (s, 5H, Ph), 7.62 (d, 2H, J=8Hz, protons m- to nitro substituent), 7.76 (d, 2H, J=8Hz, protons o- to nitro substituent), 8.24 (m, 1H, N=C<u>H</u>)
m/z (70eV EI) 298 (M^+), 193 (loss of NCH_2Ph), 162 (loss of $CH_2C_6H_5NO_2$).
Difficulty was encountered in obtaining a satisfactory analysis for this compound, due to its lack of stability.

<u>Preparation of t-butyl(2-benzyl-6β-phenoxyacetamido-2-aza-1-thiapenicillanate) (329).</u>

δ (d^8 THF, major isomer) 1.47 (s, 9H, ^tBu), 4.04 (d, 1H, J=6Hz, $NC\underline{H}_APh$), 4.17 (d, 1H, J=6Hz, $NC\underline{H}_BPh$), 4.60 (d, 1H, J=11Hz, $PhOC\underline{H}_A$), 4.66 (d, 1H, J=11Hz, $PhOC\underline{H}_B$), 5.15 (dd, 1H, J_1=5Hz, J_2=8Hz, 6-H), 5.23 (d, 1H, J=5Hz, 5-H), 6.9-7.9 (m, 10H, aromatics), 8.56 (d, 1H, J=8Hz, CONH).
m/z (+ve FAB) 470 (M^++1), 442 (loss of CO), 438 (loss of S), 413 (loss of ^tBu), 378 (loss of $PhCH_2$), 369 (loss of $CO_2{}^tBu$), 312, 222, 207, 164, 115, 106, 91..
Difficulty was encountered in obtaining a satisfactory analysis for this compound, due to its lack of stability.

Preparation of t-butyl oxamate (335).

m.p. (toluene) 83-86°C (lit[167] 89.5-90.5°C).

Preparation of t-butyl cyanoformate (333).

b.p. 30°C (35mmHg). (lit[167] 64-65°C (55mmHg)).

Preparation of benzyl thioxamidate.

mp. 86°C
δ (60MHz, $CDCl_3$) 5.25 (s, 2H, $C\underline{H}_2Ph$), 7.35 (s, 5H, aromatics), 8.20 (bs, 2H, NH_2).
^{13}C nmr ($CDCl_3$) 187 ($\underline{C}SNH_2$), 159 ($\underline{C}O_2CH_2Ph$), 134.3 (aromatic C bonded to CH_2), 128.7 and 128.4 (aromatics), 69.3 ($\underline{C}H_2$).
m/z (EI) 195 (M^+), 167.

Preparation of benzyl thiooxamimidate (336, R=Bz).

mp. 78-82°C, dec. (lit[168] 75-83°C).
δ (60 MHz, $CDCl_3$) 1.40 (t, 3H, $C\underline{H}_3CH_2$), 3.35 (q, 2H, $CH_3C\underline{H}_2$), 5.4 (s, 2H, $C\underline{H}_2Ph$), 7.3 (s, 5H, aromatics), 9.55 (bs, 2H, $C{=}N\underline{H}_2$).

Preparation of t-butyl thioxamidate.

mp. 78°C
δ (60MHz, $CDCl_3$) 1.55 (s, 9H, ^{t}Bu), 8.1 (bs, 2H, $CSN\underline{H}_2$).

Preparation of t-butyl thioxamimidate (336, R=^{t}Bu).

mp. 66-69°C, dec.
δ (60MHz, $CDCl_3$) 1.4 (t, 3H, $C\underline{H}_3CH_2$), 1.55 (s, 9H,

^{t}Bu), 3.35 (q, 2H, $CH_3\underline{CH_2}$), 9.4 (bs, 2H, $C{=}N\underline{H_2}$).

Preparation of triethyloxonium tetrafluoroborate.

mp. 89° C (dec.) (lit[169] 91-92° C)

Preparation of 3-(p-nitrobenzyl)-[7β-phenoxyacetamido-2-aza-1-thiapenem] (343).

δ (270MHz, $CDCl_3$) 4.52 (d, 1H, J=15Hz, $PhOC\underline{H_A}$), 4.62 (d, 1H, J=15Hz, $PhOC\underline{H_B}$), 5.46 (d, 1H, J=11Hz, $C\underline{H_A}C_6H_4NO_2$), 5.60 (d, 1H, J=11Hz, $C\underline{H_B}C_6H_4NO_2$), 5.52 (dd, 1H, J_1=15Hz, J_2=4Hz, 6-H), 5.93 (d, 1H, J=4Hz, 5-H), 6.8-8.3 (m, 9H, aromatics and CONH).

m/z No molecular ion was observed with either +ve or -ve FAB, or with EI or CI. This is thought to be due to the lack of stability of this compound. Some fragment ions were noted with +ve FAB: 409, 395, 391, 350 (loss of $PhCH_2$), 322 (loss of CO from m/z 350), 277 (loss of CO_2PNB), 207 (due to cleavage of C6-C7, C5-N4 and S1-N2).

Difficulty was encountered in obtaining a satisfactory analysis for this compound, due to its lack of stability.